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The influence of wood species of casks on matured whisky aroma

Identification of unique character imparted to Whisky by casks constructed of Japanese oak

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December 2016

Abstract

Traditionally, whisky is matured in casks made of oak and the aroma is greatly influenced by wood species. Currently, the predominant cask types used are those of either American or European oak. In Japan, in addition to these types of cask, a small number of Japanese oak casks have also been used. Maturation in Japanese oak is known to give unique coconut aromas.

The aim of this research was to identify the unique coconut aromas imparted by Japanese oak. The sensory properties of Japanese oak whiskies were investigated in comparison with whisky matured in American and European oak casks with particular attention to lactone isomers known to have a coconut aroma. Whiskies matured in Japanese oak casks were found to develop substantially higher levels of *trans*-lactone relative to whiskies matured in either American or Spanish oak. This research focused on the influence of lactone isomers and found the possibility of a synergistic effect between the isomers on coconut aroma. Further investigation on the impact of heat treatment on regeneration of casks determined that colour and aromatics were generated, while lactones were not regenerated once the levels had been depleted by a period of maturation.

Acknowledgements

I wish to thank Professor Alex Speers, Dr. Fergus Priest, Dr. Paul Hughes, Dr. John Conner, Dr. Frances Jack, Dr. Dawn Maskell, and Dr. Annie Hill my supervisors for their advice and encouragement throughout the course of this project. I would like to thank SWRI members for their cooperation on my sensory analysis. I would like to express my thanks the ICBD staff at Heriot-Watt University for their support during my experimental work. Thanks are also due to Ms. Minako Koike and other colleagues at Suntory Spirits Limited for their analytical help.

Suntory Spirit Limited is thanked for its financial support.

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Chapter 1: Introduction

1.1 Whisky production

1.1.1 The whisky making process

Whisky is categorised by both its production location and process into five types: Scotch whisky, Irish whiskey, Bourbon whiskey, Canadian whiskey, and Japanese whisky. Within each category the grain of malted barley, maize, wheat, or a mixture of these is used as a raw material.

As a typical example of the whisky making process, the production of Scotch, Irish, or Japanese malt and grain whisky is described in **Figure 1.1** (Noguchi, 2009). Firstly in the case of malt whisky, barley is malted in a three-step process (steeping, germination and drying) to activate enzymes present in the barley grain and breakdown starch reserves. Next, malted barley is milled into grist and mixed with hot water in order to obtain wort. Yeast is added to the wort and fermentation is processed. After fermentation, about seven to nine % abv wash is obtained. Typically wash is distilled twice or three times (especially in Irish whisky) by pot stills made of copper and new make spirit, which is clear and colourless, and has strength of about 70 - 80% abv. On the other hand, for grain whisky, maize or wheat are mainly used, and distillation is carried out by continuous stills in order to obtain clearer new make spirits. The distillates are diluted by water down to approximately 60% abv, and filled in casks made of oak. The spirit develops colour during maturation or by use of caramel.

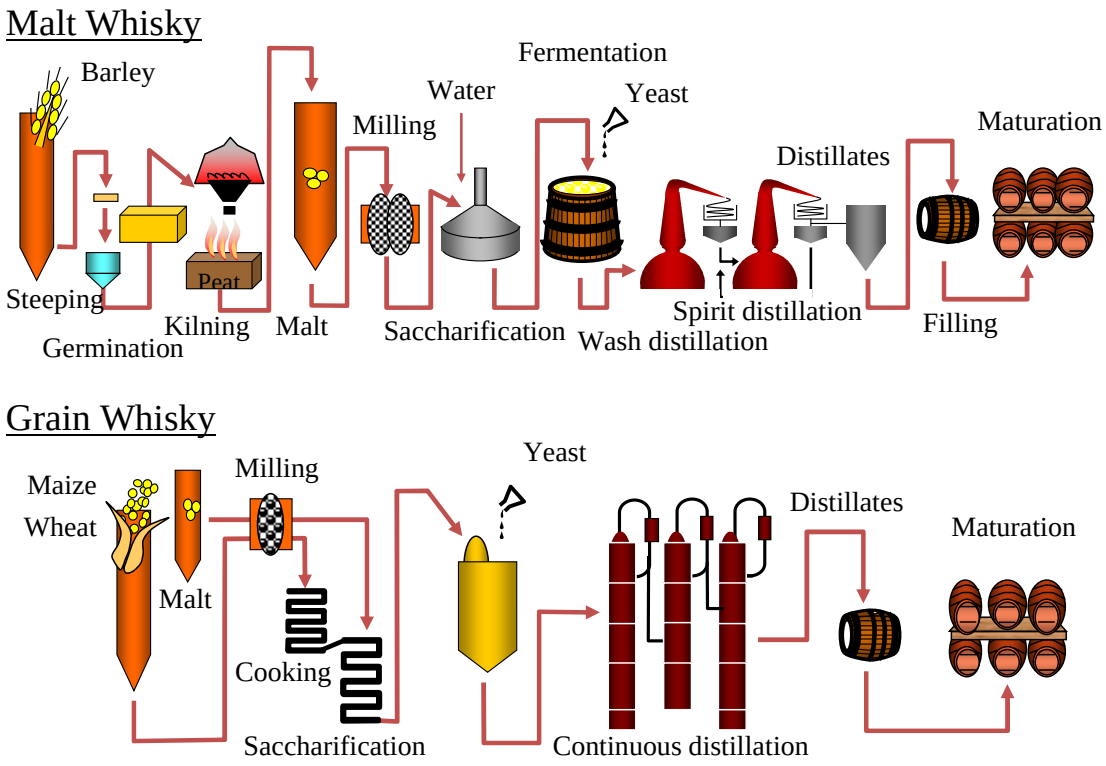


Figure 1.1 Whisky making processes (Noguchi, 2009)

1.1.2 Whisky maturation

During maturation both alcoholic and harsh tastes reduce and woody and sweet aroma increases. This process is one of the most important processes required to form whisky aroma. Maturation period depends on target quality, but is generally at least three years (the minimum maturation period required for Scotch whisky (Scotch Whisky Regulations, 2009)). A cask is not an airtight container, but lets through air, alcohol, or water gently, so that oxidation by air or evaporation of alcohol takes place during this process. This process is very complicated and scientific explanation of the maturation is not enough, but the mechanism is broadly understood as five primal points that influence aroma formation.

1. Decomposition and extraction of wood components.
2. Oxidation of components in new make spirit.

3. Generation of aromatic compounds by acetalization and esterification.
4. Association of water and alcohol molecules.
5. Evaporation of alcohol and water: concentration of non-volatile compounds.

These five points are described below.

1.1.2.1 Decomposition and extraction of wood components

Wood components mainly consist of cellulose, hemi-cellulose, and lignin, and hydrolyzed or pyrolized decompositions formed by charring the inside of the cask are extracted to whisky. Many of the decompositions derived from lignin are aroma active compounds such as vanillin, which imparts a sweet aroma. In addition to vanillin, syringaldehyde, and coniferaldehyde are also extracted. These compounds are generated by charring (**Table 1.1**; Nishimura et al., 1989; Baldwin et al., 1967).

	Barrel type		
	New uncharred	Used	New charred
Barrel proof	136.7	137.0	140.8
Age, years	6	6	1.5
Aromatic aldehyde, g/100 °proof			
Coniferaldehyde	0.03	0.03	0.12
Sinapaldehyde	0.02	0.04	0.13
Syringaldehyde	0.12	0.14	0.45
Vanillin	0.12	0.06	0.07
Total	0.29	0.27	0.77

200 °proof = 100% abv

Table 1.1 Aromatic aldehydes during maturation reported by Nishimura et al., (1989)

In addition to these compounds, many compounds derived from the cask have been identified. One of the well-known compounds is whisky lactone, which has a significant influence on whisky aroma (Masuda et al., 1971; Suomalainen et al., 1970).

1.1.2.2 Oxidation of components in new make spirits

Cask breathe through the wood stave and air comes into the cask. Therefore, components of spirits are gently oxidized. Since the surface of casks are normally charred, oxidation reactions on the char surface are promoted and adsorption to char also occurs. Ethanol is oxidized into acetaldehyde or acetic acid. This oxidation reaction was observed by an experiment using ethanol marked by labelled carbon (^{14}C; Reazin et al., 1976). Dimethyl sulphide, which is a compound of unpleasant seaweed aroma, is oxidized into Dimethyl sulphoxide. This reaction is promoted by char (Fujii et al., 1992).

1.1.2.3 Generation of aromatic compounds by acetalization and esterification

Aldehydes and esters are groups of flavour compounds contained in whisky. They have a low aroma threshold and are one of the prime aroma compounds in whisky. Aldehydes generally have stimulative aroma, but acetals have pleasant fruity aroma (Perry et al., 1986). Many aldehydes change into acetals by static reactions during maturation (**Figure 1.2**). When the level of aldehydes and acetals before maturation are compared with after four or five years maturation, the observed trend is that aldehydes, such as diacetyl, decrease and acetals increase (Masuda et al., 1980; Reazin et al., 1981). On the other hand, esters have fragrant fruity aromas and they are increased during maturation because carboxylic acids esterify with ethanol. Most of this esterification is ethyl acetate from acetic acids (Reazin et al., 1981).

$$C_2H_5OH + CH_3CHO \rightarrow CH_3CHOC_2H_5 + C_2H_5OH$$

$$\downarrow$$

$$CH_3CH(OC_2H_5)_2 + H_2O$$

Figure 1.2 Acetal formation from ethanol and acetaldehyde (Koga, 2009)

1.1.2.4 Association of water molecules and alcohol molecules

In addition to various chemical reactions, physical changes also occur during maturation. A representative example is the change of the association of water molecules and alcohol molecules. The association has been measured by various methods including thermodynamic calculation of hydrogen bonding, mass spectrometry of liquid cluster, and small angle X-ray scattering (Nishimura et al., 1983; Aishima et al., 1992; Furusawa et al., 1990). It is thought that alcohol harshness generally weakens due to these changes and they contribute to a smooth taste (Nishimura et al., 1989).

1.1.2.5 Evaporation of alcohol and water: concentration of non-volatile compounds

Volatile compounds including ethanol and water evaporate little by little through wood during maturation. The evaporation ratio of various compounds was measured in laboratory scale experiments by Hasuo et al. (1986) using ethanol model solutions (**Table 1.2**). Acetaldehyde has a high ratio, but acetic acid low (Hasuo et al., 1986). The evaporation ratio is normally influenced by a compound's boiling point, but also influenced by both the degree of penetration into the cask and volatility. Since acetic acid is generated from the decomposition of wood, the calculation of evaporation needs consideration of wood extraction. In the case of ethyl caproate, this is a low volatile compound and the loss is less than one percent of total liquid, even excluding evaporation.

Low volatile or non-volatile compounds are concentrated as a result. When the volume is decreased and headspace in the cask increased through maturation, air comes into the cask and oxidation reactions are promoted (Conner et al., 2003).

	Evaporation ratio		
	3 months	6 months	12 months
Acetaldehyde	4.21	10.82	31.98
Ethyl alcohol	1.68	4.26	12.66
n-Propyl alcohol	1.47	3.45	10.59
Ethyl acetate	1.34	2.76	10.49
Isobutyl alcohol	1.07	2.54	8.66
Isoamyl acetate	0.67	1.74	5.35
Isoamyl alcohol	0.62	1.65	5.15
Ethyl caproate	0.20	0.58	1.28
Acetic acid	0.25	0.28	0.96
Water	1.71	7.76	7.59
Total	1.69	3.52	10.17

$$\text{Evaporation ratio} = \frac{\text{Amount of the substances evaporated from barrel of whisky}}{\text{Amount of the added substances}} \times 100$$

Table 1.2 Volatile ratio of compounds in ethanol model solution reported by Hasuo et al. (1986)

As described above, changes during maturation are influenced by the balance of chemical reactions and physical reactions. Therefore, even if one factor is changed, significant changes on aroma or quality can occur; there are many factors, chemical components in spirit, condition of maturation, oak species, cask size, charring condition, etc. In whisky production, whisky is made with great attention to various factors in order to control its aroma and quality (Nishimura et al., 1989).

1.2 Whisky casks

Traditionally whisky is matured in casks made of oak. In the scientific classification, oak belongs to the family *Fagaceae* in the order *Fagales*, and the Genus is *Quercus*. The aroma of the maturing spirit is influenced by both wood species and previous cask history (Conner et al., 2003). Currently, bourbon casks made of American oak (*Quercus alba*) which has been previously used for bourbon whiskey are mainly used. These casks impart a light and floral aroma to a whisky (Conner et al., 2003). Alternatively, sherry, brandy, or French wine casks, made of European oak (*Quercus robur* or *Quercus sesslilis*) that have been previously used for sherry, brandy, or French wine maturation are used. These are quite different from American oak, with spirit matured in these casks containing higher levels of colour and cask extractives (Conner et al., 2003). Sherry casks, made from European oak, produce whiskies with typical 'sherry wood whisky' characteristics, combining vanilla, fruity and sweet aromas (Conner et al., 2003). Both types of cask are used in Japan to mature malt and grain spirits. However, for more than 50 years, a small number of casks made from Japanese oak (*Quercus mongolica*) have also been used. Maturation in Japanese oak casks is known to give unique aromas, 'Japanese shrine and temple', or 'heavy oriental smell' which has never seen in other kinds of oak (Koshimizu, 2011).

Oak wood is normally used to construct casks for whisky maturation; indeed, the Scotch Whisky Regulations strictly stipulate its use (Scotch Whisky Regulations, 2009). However, other wood varieties are used for other alcohol beverages. Chestnut (*Catanea aiva*) had historically been used for wine maturation as a good substitute for oak (DeRosso et al., 2009). In addition to chestnut, cherry (*Prunus avium*), acacia (*Robinia pseudoacacia*), and mulberry (*Morus alba* and *Morus nigra*) are known as a wood for

casks and the wines matured in these casks were reported to have different aroma profiles (Fernández de Simón et al., 2014). In the case of cherry, this was most oxidative environment in these varieties, and therefore the least suitable for long term maturation (DeRosso et al., 2009).

Casks are made by a combination of staves. In the case of barrels, around 30 staves are required (**Figure 1.3**), and in the case of butts, around 50 staves (Conner et al., 2003). When spirits are charged in the cask, maturation is started and various changes occur (**Section 1.1**). Whisky is evaporated and its volume decreases during maturation. As a result, it is assumed that the top stave would become dry over time as it has reduced contact with the liquid, the bottom stave would be wet, with some staves in the top third of the cask having shortened contact as evaporation reduces the liquid level.

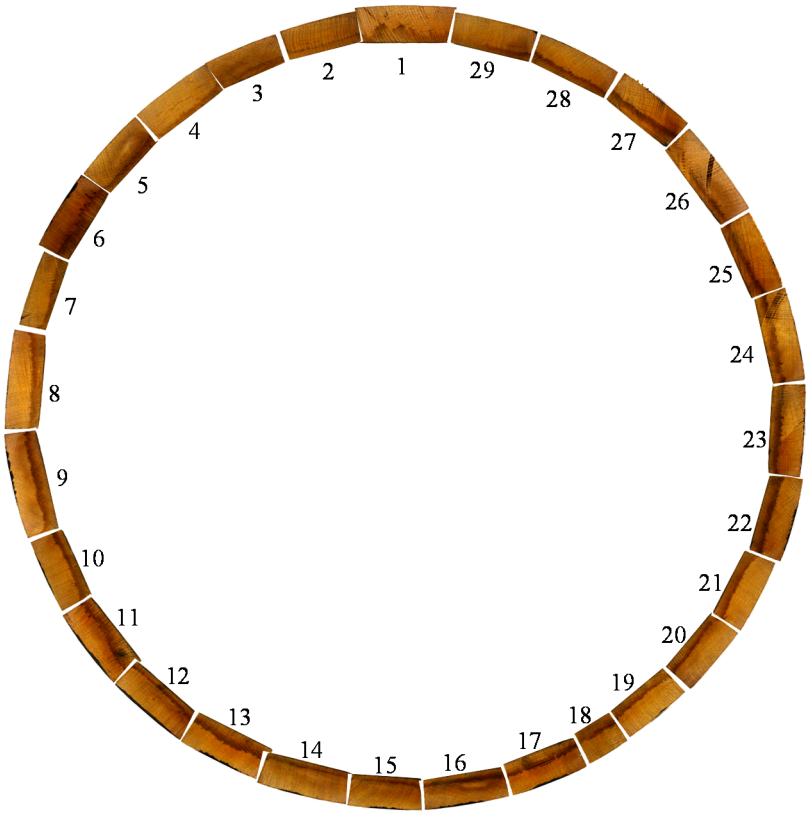


Figure 1.3 Example of the combination of staves for barrel.

1.3 Cask regeneration

Whisky casks are normally used for a couple of decades. When the whisky is discharged after a maturation period, the casks are reused until the cask is exhausted and fail to add a aroma or colour to whisky. These casks can be regenerated by de-charring the inner face and re-charring using a gas burner. When casks are re-charred, thermal degradation of lignin yields aroma compounds similar to those produced in a new charred cask. However, the balance of wood extractives in regenerated casks is very different from that of a new charred cask (Conner et al., 2014).

1.4 Japanese oak casks

Japanese oak is called ‘Mizunara’ in Japanese, which means water oak. The habitat of Japanese oak is East Asia, including Japan, the Korean Peninsula, north east of China, and south Sakhalin, and its formal scientific name is *Quercus mongolica Fischer ex Turczaninow varietas grosseerrata (Blume) Rehder et Wilson*. The characteristics of this oak are that it is softer and has fewer tyloses than American oak (Kato, 1985). Japanese oak casks, of 500 litre capacity, are generally seasoned with oloroso sherry type wine for one year. After seasoning, the cask is used for whisky maturation. At a younger age the Japanese oak matured whisky has fresh and light characteristics, similar to those obtained using a bourbon cask. However, over twenty years, the aroma changes into a unique one, which is different from that produced by other oak casks. In Japan these aromas are described as ‘well ripened pineapple’, ‘melted butter and cinnamon’, ‘Japanese shrine and temple’, and ‘heavy oriental smell’ (Koshimizu, 2011; Noguchi et al., 2008).

1.5 Objectives

As described above, since the whisky matured in Japanese oak cask has unique aromas, the aim of this research was to identify the unique aromas imparted by casks of Japanese oak. Here, the sensory properties of these Japanese oak whiskies, relative to the other whisky types was studied. In addition to this, observations regarding the impact of oak species on the isomeric ratio of whisky lactones were studied, which are known to make an important contribution to mature character (Masuda and Nishimura, 1971). Whiskies matured in Japanese oak casks for up to forty years develop substantially higher levels of *trans*-whisky lactone, relative to whiskies matured in either American or Spanish oak. This raises the possibility that the whiskies matured in Japanese oak can be distinguished analytically, by the determination of the whisky lactone ratio and content.

Chapter 2: Materials and methods

2.1 Samples

2.1.1 Whisky samples

Whiskies (**Table 2.1**) were used for the sensory analysis (**Section 2.4**) and the chemical analysis (**Section 2.3**).

		Sample Name	Whisky Type	Spirit	Distilled Year	Strength [% abv]	Cask Treatment
Japanese oak whisky	Young whisky	JPN-9yo	Malt	Yamazaki	2000	56.0 - 59.8	No treatment
		JPN-10yo	Malt	Yamazaki	1999	56.3 - 59.8	Re-charred
	Old whisky	JPN-20yo	Grain	Chita	1987	47.6	
		JPN-27yo	Malt	Yamazaki	1980	63.0	
		JPN-40yo	Malt	Yamazaki	1960	58.2	
American oak whisky		USA-20yo	Grain	Chita	1988	52.9	
		USA-27yo	Malt	Yamazaki	1980	53.7	
		USA-40yo	Malt	Yamazaki	1968	63.7	
Spanish oak whisky		SPN-20yo	Grain	Chita	1988	54.5	

Table 2.1 Whisky samples

These were selected as examples of typical matured whiskies of each age. Throughout this thesis, the whiskies are referred to as;

- ‘JPN whisky’ referring to whisky matured in casks made of Japanese oak, *Quercus mongolica Fischer ex Turczaninow varietas grosseerrata (Blume) Rehder et Wilson*
- ‘USA whisky’, that is whisky matured in casks made of American oak, *Quercus alba*
- ‘SPN whisky’, whisky matured in casks made of Spanish oak, *Quercus robur* or *Quercus petraea*

It was assumed that old whiskies (20 - 40 years) of USA and SPN origin would approximately correspond in age to JPN whiskies for comparison purpose. Young JPN whiskies from the same malt distillery, but different cask treatments, were selected were also used in this study.

2.1.2 Wood samples

Staves were collected from casks in which whisky (as described in **Table 2.1**) was matured or supplied from a cooperage (Omi Cooperage Limited, Japan), were cut into slices using a wood slicer (Electric planer, MAKITA, Japan) in the cooperage. A series of 1 mm slices were collected and these slices were then cut into chips by the cooperage (**Figure 2.1**).

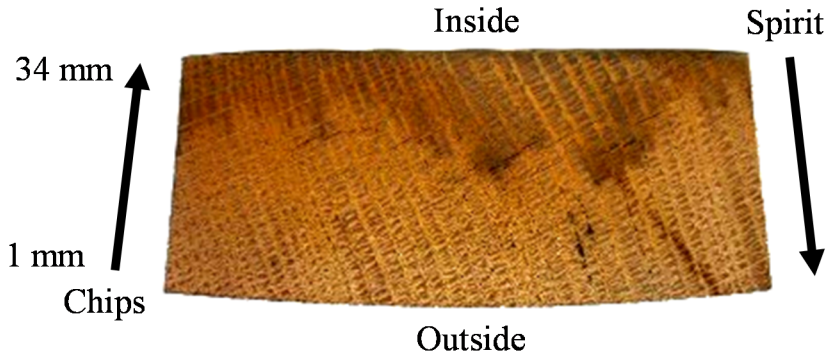


Figure 2.1 Wood sample collection

2.1.3 Whisky lactones

The whisky lactone used in this study was obtained from Sigma Aldrich (Dorset, UK), this was reported to contain the same ratio of the four isomers subsequently listed (Guichard et al., 1995). The isomers of this reagent were separated to both *cis*-lactones, 3S,4S (*cis*) and 3R,4R (*cis*), and both *trans*-lactones, 3S,4R (*trans*) and 3R,4S (*trans*), by silica-gel chromatography (**Section 2.2.5**). The purities of these separated lactones were quantified by GC-MS (**Section 2.3.5**). No *cis*-lactones were detected in the separated *trans*-lactones but the *cis*-lactones contained 1.1% of *trans*-lactones. This contamination level is thought to be low enough to disregard. When the separated *cis*-lactones and *trans*-lactones, which include the natural and unnatural lactones, were compared with the natural lactones in whisky using GC-Olfactometry (**Section 2.3.5**), the separated lactones showed almost the similar aroma to the natural lactones which described 'heavy & oily' as *cis*-lactone and 'light & fresh' as *trans*-lactone refer to sensory panels. Thereby, these separated lactones were used respectively as *cis* or *trans*-lactone for the duration of this study.

2.1.4 Ethanol solutions

Ethanol solutions (20, 40, 60, 80% abv) were prepared by dilution of 99.5%+ ethanol obtained from Kanto Chemical (Tokyo, Japan) with pure water obtained from a Milli-Q integral system (Millipore, Tokyo, Japan).

2.1.5 Model ethanol solutions

The model solutions were prepared, each contained one of the lactones (*cis* or *trans*) which was prepared using the separation method described in **Section 2.2.5**. Lactones were added to give final concentrations of 0.25, 0.50, 0.75, 1.00 mg/L respectively or other concentrations in a 20% abv ethanol solution (**Section 2.1.4**).

2.1.6 Whisky samples with additional lactones

Whisky samples of 10 mL volume (**Table 2.1**) were prepared, to these 0.1 mL of 100 mg/L lactone ethanol solution (**Section 2.2.5**) was added. The two lactone isomers (*cis* and *trans*) were added individually.

2.2 Experiments

2.2.1 Extraction from wood chip

For the chemical analysis of woods, wood components were extracted from wood chips by stirring these with 20 mL of a 60% abv ethanol solution (**Section 2.1.4**). The quantity of wood chips used was 2.0 g (**Section 2.1.2**) and extractions occurred for one day (24 hours) at room temperature (approximately 20°C).

2.2.2 Heat treatment of wood chips

Samples of wood chips (4.0 g) were placed on a petri dish and heated in a gas chromatography oven (HP 5890 series, Agilent, UK), for two, four, and six minutes at 130, 150, 180, 210, 250, and 300°C respectively. After heating, these chips were cooled naturally and stored in plastic bags in order not to be affected by heat again.

2.2.3 Heat treatment of the stave

The charring of staves was carried out using the handy burner of GB-2001 (Prince, Japan). The heating strength of light or heavy charring was determined by visual observation. The toasting of staves was carried out by placing on the hot plate of PC-420D (Titec, Japan) at 250°C for 30 minutes.

2.2.4. Colour measurement of extracted solution

The colour of the extracted solution was measured by the attenuation of light at 430 nanometres using a UV-Visible Spectrophotometer (Shimadzu, Japan) using the European Brewery Convention method (EBC Method 9.6, 2000).

2.2.5. Separation of whisky lactone isomers

The reported method (Otsuka et al 1974) was used for the separation of lactone isomers. Using 40 mL of silica-gel 70 - 230 mesh (Sigma Aldrich, Dorset, UK) the cylinder column (ϕ = 1 cm) was filled. The mixture solvent of diethyl ether - pentane (1:6) obtained respectively from Fisher Scientific (Hull, UK) was passed through the column until

remained air was removed. Following this 200 mg of whisky lactone mixture (Sigma Aldrich, Dorset, UK) was added to the column and the solvent of ether - pentane (1:6) was flown. The flown solvent was collected as 32 fractions of 10 mL. The 12 - 14 fractions for *trans*-lactone and the 25 - 29 fraction for *cis*-lactone fraction were concentrated in about 40°C hot water on an individual basis. To these concentrated fractions 99.5%+ ethanol (Fisher Scientific, Hull, UK) was producing a 100 mg/L of lactone ethanol solution.

2.3 Chemical Analysis

The analysis of general volatile compounds in whisky (Piggott et al., 1993), specifically to determine the concentration of fatty acids (**Section 2.3.1**), fatty acid esters (**Section 2.3.2**), and fusel alcohols (**Section 2.3.3**) was carried out by gas chromatography. The concentrations of these components were calculated as 100% abv, this was because these compounds are derived from the original spirit (metabolites of fermentation) and were evaporated during maturation. The analysis of general wood extractives of aromatic compounds in whisky were carried out with HPLC (**Section 2.3.4**). The analysis of whisky lactones were carried out with GC-MS (**Section 2.3.5**). The concentrations of aromatics and lactones were calculated on an 'as is' basis.

2.3.1 Fatty acids

Analysis was carried out on a GC-FID, Shimadzu 2010 (Shimadzu, Japan). The column was a 50 m x 0.32 mm HP-FFAP capillary column with a film thickness of 0.5 μm (Agilent, Japan). The initial oven temperature was 100˚C, increasing to 210˚C at

5˚C/min with a final hold time of 28 min. The injector temperature was maintained at 250˚C. The fatty acids compounds which were analysed for were acetic acid, propionic acid, iso-butyric acid, iso-valeric acid, hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid. Data were collected using the GC solution (Shimadzu, Japan).

2.3.2 Fatty acid esters

Analysis was carried out using a GC-FID, 6890N (Agilent, Japan). The column was a 50 m x 0.32 mm HP-ULTRA2 capillary column with a film thickness of 0.52 μm (Agilent, Japan). The initial oven temperature was 42˚C with a hold time of 8 min, increasing to 230˚C at 10˚C/min with a final hold time of 23 min. The injector temperature was maintained at 250˚C. The fatty acid ester compounds which were analysed for were ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl hexadecanoate, and ethyl hexadecenoate. Data were collected using the Chemstation (Agilent, Japan).

2.3.3 Fusel alcohols

Analysis was carried out on a GC-FID, 6890N (Agilent, Japan). The column was a 50 m x 0.32 mm HP-ULTRA2 capillary column with a film thickness of 0.52 μm (Agilent, Japan). The initial oven temperature was 42˚C with a hold time of 8 min, increasing to 230˚C at 10˚C/min with a final hold time of 23 min. The injector temperature was maintained at 250˚C. The compounds of fusel alcohols compounds which were analysed for were n-propyl alcohol, iso-butanol, iso-amyl alcohol, active-amyl alcohol, and β-phenylethyl alcohol. Data were collected using the Chemstation (Agilent, Japan).

2.3.4 Aromatic compounds

Analysis was carried out using a HPLC, LC-10AD (Shimadzu, Japan) with the UV detector, SPD-10A (Shimadzu, Japan). The column was a Shim-pack CLC-ODS 0.15 m x 6.0 ϕ column (Shimadzu, Japan). The oven temperature was 50˚C. Aromatics analysed for were vanillic acid, syringic acid, vanillin, syringaldehyde, and sinapaldehyde. Data were collected using the LC solution (Shimadzu, Japan).

2.3.5 Whisky lactones

Analysis was carried out using a GC, Hewlett-Packard 5890 series II (Agilent, UK). The flow from the GC was split in the approximate ratio 6:1 between a Gerstel ODP2 olfactory detection port fitted with a glass sniffing cone (GERSTEL, Germany) and a 5971 mass spectrometer (Agilent, UK). The column was a 60 m x 0.32 mm ZB-Wax capillary column with a film thickness of 0.5 μm (Phenomenex, UK). The initial oven temperature was 80˚C, increasing to 250˚C at 3˚C/min with a final hold time of 5 min. The injector temperature was maintained at 250˚C. The mass spectrometer was operated in the electron impact (EI) mode and ions from 99 amu were scanned. Data were collected using the Chemstation (Agilent, Japan).

2.4 Sensory analysis

2.4.1 Sensory panellists

Sensory experiments were carried out by the Scotch Whisky Research Institute's internal sensory panel. This panel compromised of 20 highly trained members of staff who had undergone extensive sensory training and with substantial experience in the assessment of alcohol beverages. A minimum of seven panelists participated in each sensory session. Assessments were carried out in individual booths.

2.4.2 Sample preparation and presentation

Whisky samples as described in **Table 2.1** were reduced to 20% abv using well run tap water. A total sample size of 20 mL was presented for assessment in clear colourless 130 mL nosing glass, and were covered with 50 mm watch glasses in order to retain any headspace volatile compounds. Samples were identified using three random codes and presentation order was randomized. Sensory panelists performed the assessment in individual booths, under red light, to overcome any bias relating to sample colour or turbidity. All assessments were based on nosing (aroma) only, with no tasting being carried out as part of this work. Data were collected using Compusense V.5, sensory data collection software (Compusense Inc., Canada)

2.4.3 Quantitative descriptive analysis

All sensory analysis was carried out using Quantitative Descriptive Analysis, which was used to provide a measure of the relative intensity of a range of pre-determined sensory

attributes. This test was set-up in accordance with British Standard BS 13299:2003 (BS 2003). Attributes were scored using a scale of 0.0 to 3.0 with intervals of 0.1. Data were collated and exported to Excel and examined for significant differences (**Section 2.5**).

2.4.4 Threshold measurement

The threshold measurement was determined by following the method described in ISO 13301 which is classified as 'general guidance for measuring odour, aroma, and taste detection thresholds by a three-alternative forces-choice (3-AFC) procedure'. The samples were prepared in 20% abv ethanol solution, which included each concentration of lactone or lactones, *cis*-lactone (**Table 2.2**).

	Concentration [mg/L]					
cis - lactone	0.00	0.05	0.10	0.15	0.20	0.25
trans - lactone	0.00	0.40	0.80	1.20	1.60	2.00
Mixture (*cis* :*trans* = 1:1)	0.00	0.05	0.10	0.15	0.20	0.25

Table 2.2 Lactone concentrations for threshold measurement in 20% abv ethanol solution

If an aroma could not be detected by any of the panellists the data point was removed before carrying out the statistical analysis. The total number of panellist against log concentration was plotted, and a straight line was fitted to the graph following the standard methodology described in ISO 13301. The formula of this line, $y = \mathrm{A}x + \mathrm{B}$,

was calculated by the least squares method. The Log concentration at which half of the panel can detect the aroma was calculated;

$$\text{Log concentration} = \frac{(H - B)}{A}$$

H = half the total number of panellists

The log concentration was converted back to standard concentration of mg/L.

2.5 Statistical analysis

Sensory and chemical analysis data were analysed by determining whether or not the number was significantly greater than the number likely to be obtained. This analysis was carried out using a tool of Microsoft Excel software (Washington, USA). A probability value (p value) of <0.05 for repeated data by t-test which is generally used for comparison of two samples or ANOVA (Analysis of variance) which is generally used for more than 3 samples were interpreted as significant difference.

Chapter 3: The influence of wood species of cask on matured whisky aroma

3.1 Introduction

Whisky which has been matured in Japanese oak casks for more than 20 years, has often been described by consumers as having a unique aroma, which is not found in whisky that has been matured in casks constructed from other types of oak. These aromas have been described by Japanese consumers as, 'Japanese shrine and temple', 'heavy oriental smell', it has been suggested that these terms describe the Japanese taste (Koshimizu, 2011; Noguchi et al., 2008).

Whiskies matured in Japanese oak cask have recently drawn attention from the whisky industry (Shimatani et al., 2013) and these whiskies have won awards in worldwide competitions. Recently, in the International Spirits Challenge, Yamazaki Mizunara cask whisky was awarded the Gold Medal three times between 2013 and 2015 (it should be noted that Mizunara means Japanese oak in Japanese). Therefore, whiskies matured in Japanese oak casks have the potential to be in greater consumer demand in the future. However, the terminology used to describe the aromas of Japanese whisky are quite 'local' terms which would be familiar to Japanese consumers, but not yet included in worldwide whisky lexicon.

The starting point for this study, as it was working with European whisky researchers, was to develop a translation of these descriptions in order to adequately share the aromas experienced. The typical aroma compounds of this whisky and associated characteristic

compounds have not previously been identified, which may be because the interest in Japanese oak whisky has only increased in recent years.

Using spirit exposed to American and European oak as a comparison, this research was designed to determine the characteristics and chemical composition of whisky matured in Japanese oak. Whiskies matured in European and American oak are those most recognised by whisky consumers around the world and the accepted terminology used in sensory analysis is often based around these (**Figure 3.1**). Finally, Japanese whisky matured in American, Spanish (European), and Japanese oak were compared by a trained sensory panellists (**Section 2.4.1**).

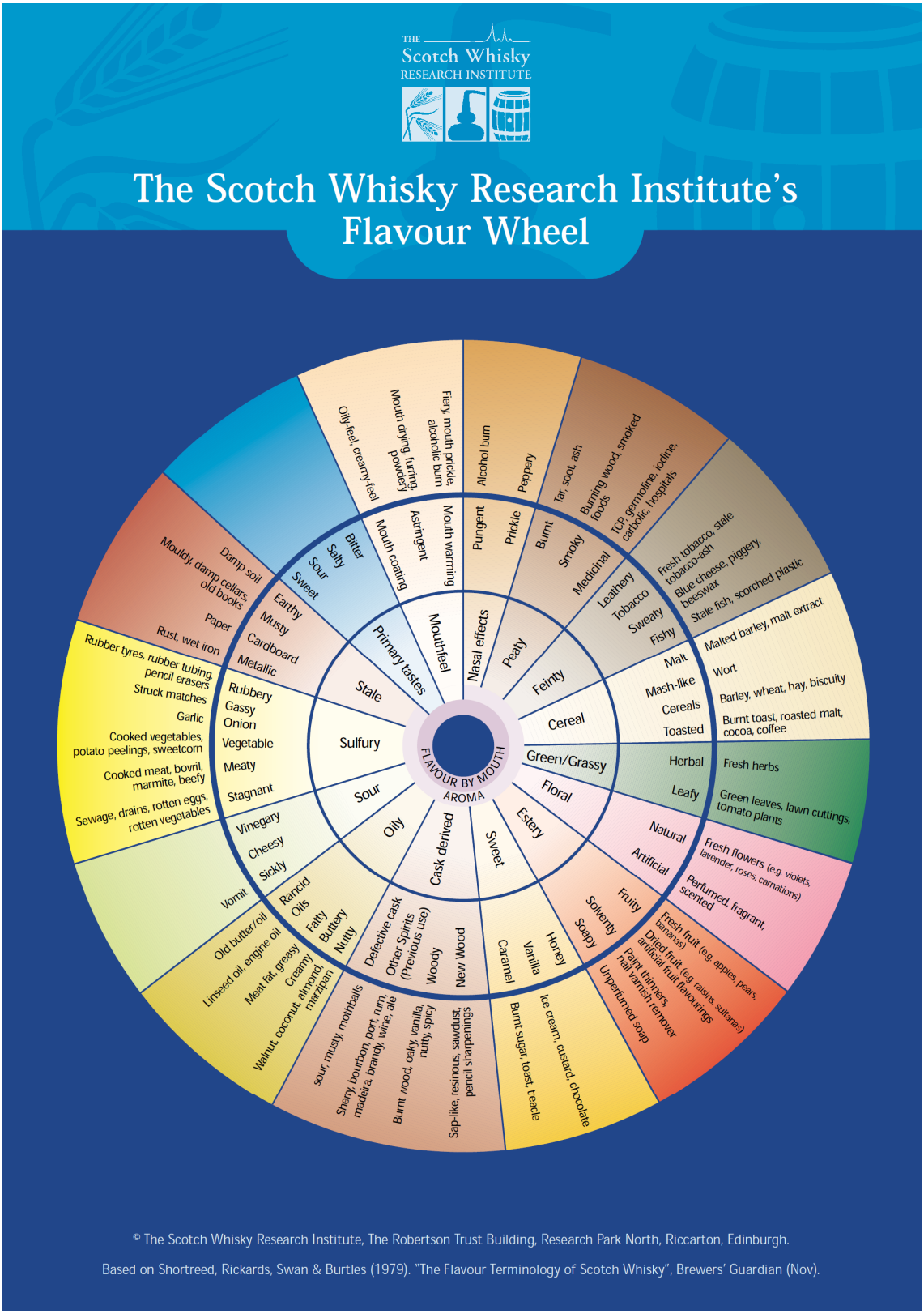


Figure 3.1 The Scotch Whisky Research Institute's Aroma Wheel

3.2 Results

3.2.1 Sensory analysis using standard aroma descriptors for the whisky industry

Sensory analysis (**Section 2.4**) was carried out by the Scotch Whisky Research Institute's internal sensory panel on a range of whiskies, those chosen for sensory analysis were a 27 year old matured in Japanese oak, and a 27 year old matured in American oak (**Section 2.1.1**). This age of matured whisky was chosen as this is estimated to demonstrate the typical aromas extracted by the spirit from each type of cask by Suntory blenders. The aroma descriptors employed are those frequently used by the whisky industry in both the United Kingdom and Japan, and which can be found on the Scotch whisky aroma wheel (**Figure 3.1**).

The individual responses generated by the sensory panel are presented in **Table 3.1**. Interestingly not all descriptors were picked up by all panel members. This is likely to represent the consumer experience, where individuals will experience whisky differently and due to differencing sensory threshold levels (Lee et al., 2000). Panellists could give scores on an increasing scale from 0.0 (low) to 3.0 (high) (**Section 2.4.3**), scores, mean scores, and standard deviations are given in **Table 3.1**, and indicate the variation which was observed between individual results.

The mean scores were compared between USA whisky and JPN whisky, and all descriptors' values were found to be similar. Since the p value (**Section 2.5**) of all descriptors with the exception of 'feinty' and 'sulphury' were more than 0.05, significant differences were not observed in the most of descriptors.

The mean scores from **Table 3.1** have been presented as a spider diagram (**Figure 3.2**). Sensory data is traditionally presented in this format which is good for visual understanding. When comparing the chosen aroma descriptors the 27 year old USA and JPN whiskies were found to be similar ($p > 0.05$) (**Table 3.1** and **Figure 3.2**). Two aroma characters were found to demonstrate significant differences between the two samples, these were the feinty ($p = 0.03$) and sulphury ($p = 0.02$) characteristics.

		Pungent	Cereal	Green grassy	Floral	Fresh fruit	Solventy	Feinty	Dried fruit	Sweet	Woody	Spicy	Oily	Struck match	Sulfury
	JPN 27yo	1.1	0.4	0.2	0.1	0.7	0.4	0.2	0.9	0.3	0.5	0.5	0.2	0.4	0.1
		1.4	1.8	2.0	2.0	2.3	1.7	2.0	2.0	1.9	1.6	2.0	0.7	0.8	0.7
		1.5	0.5	0.0	0.2	0.5	0.2	0.7	2.0	1.6	1.3	0.8	0.6	0.0	0.0
		1.3	0.2	0.8	0.5	0.7	1.2	0.8	1.3	0.7	1.3	1.1	0.5	1.2	0.3
		1.9	0.5	0.9	1.2	1.1	1.4	1.4	0.5	1.2	1.7	0.9	1.0	0.2	1.0
		0.7	0.2	0.6	0.2	0.2	1.0	0.9	1.0	0.3	1.1	0.8	1.1	0.2	0.2
		2.0	0.0	0.0	1.5	1.0	1.0	1.0	-	-	-	-	-	-	-
		1.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	0.5	0.0	0.0	0.5	1.5
		0.5	0.2	0.4	0.5	0.6	0.2	0.1	0.6	0.5	1.1	0.4	0.4	0.0	0.0
		0.1	0.0	0.2	0.5	0.0	0.0	0.0	-	-	-	-	-	-	-
		0.2	0.0	0.4	0.0	0.0	0.2	0.4	0.1	0.3	0.2	0.2	0.0	0.2	0.2
		0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-
		1.7	1.5	1.5	1.5	0.5	0.9	0.8	-	-	-	-	-	-	-
		1.7	1.0	0.8	2.0	2.0	0.6	0.5	2.3	1.5	2.5	0.5	2.0	0.8	0.4
Mean		1.1	0.5	0.6	0.7	0.7	0.7	0.7	1.1	0.9	1.2	0.7	0.7	0.4	0.4
St. Dev.		0.7	0.6	0.6	0.7	0.7	0.5	0.5	0.7	0.6	0.6	0.5	0.6	0.4	0.5
	USA 27yo	1.0	0.2	0.1	0.2	0.5	0.2	0.1	0.9	0.2	0.5	0.5	0.2	0.1	0.1
		1.1	1.7	1.7	1.8	2.1	1.7	1.5	2.2	1.6	1.6	1.7	0.8	0.5	0.5
		1.6	0.8	0.2	0.2	0.8	0.2	0.2	1.4	1.1	1.0	0.7	0.4	0.0	0.0
		1.3	0.4	1.2	1.0	1.5	0.8	0.5	1.0	1.1	1.0	0.7	0.5	0.2	0.0
		1.9	0.7	1.5	1.7	1.0	1.4	1.0	0.5	1.0	1.5	0.5	1.0	0.2	0.5
		0.7	0.2	0.6	0.2	0.2	0.7	0.5	0.7	0.3	1.0	0.5	1.1	0.4	0.2
		1.5	0.0	0.5	1.0	0.5	1.5	0.0	-	-	-	-	-	-	-
		1.5	0.5	0.0	0.0	0.5	0.0	0.5	0.5	0.5	1.0	0.0	0.0	0.5	0.5
		0.5	0.2	0.7	1.1	1.5	0.2	0.1	1.1	1.0	0.7	0.9	0.2	0.0	0.0
		0.0	0.4	0.5	0.4	0.0	0.0	0.0	-	-	-	-	-	-	-
		0.2	0.0	0.4	0.0	0.0	0.1	0.4	0.1	0.1	0.1	0.0	0.0	0.0	0.0
		1.1	0.9	1.0	0.5	1.4	1.5	1.0	-	-	-	-	-	-	-
		1.0	0.5	0.8	1.8	1.8	0.7	0.5	2.0	2.0	2.5	0.7	2.0	0.8	0.4
Mean		1.0	0.5	0.7	0.8	0.9	0.7	0.5	1.0	0.9	1.1	0.6	0.6	0.3	0.2
St. Dev.		0.5	0.4	0.5	0.7	0.7	0.6	0.4	0.6	0.6	0.6	0.5	0.6	0.3	0.2
p value by t-test (N = 7-14)		0.17	0.87	0.24	0.85	0.22	0.79	0.03	0.47	0.74	0.23	0.18	0.79	0.14	0.02

Table 3.1 Sensory analysis results for 27 year old JPN and USA whiskies using standard aroma descriptors

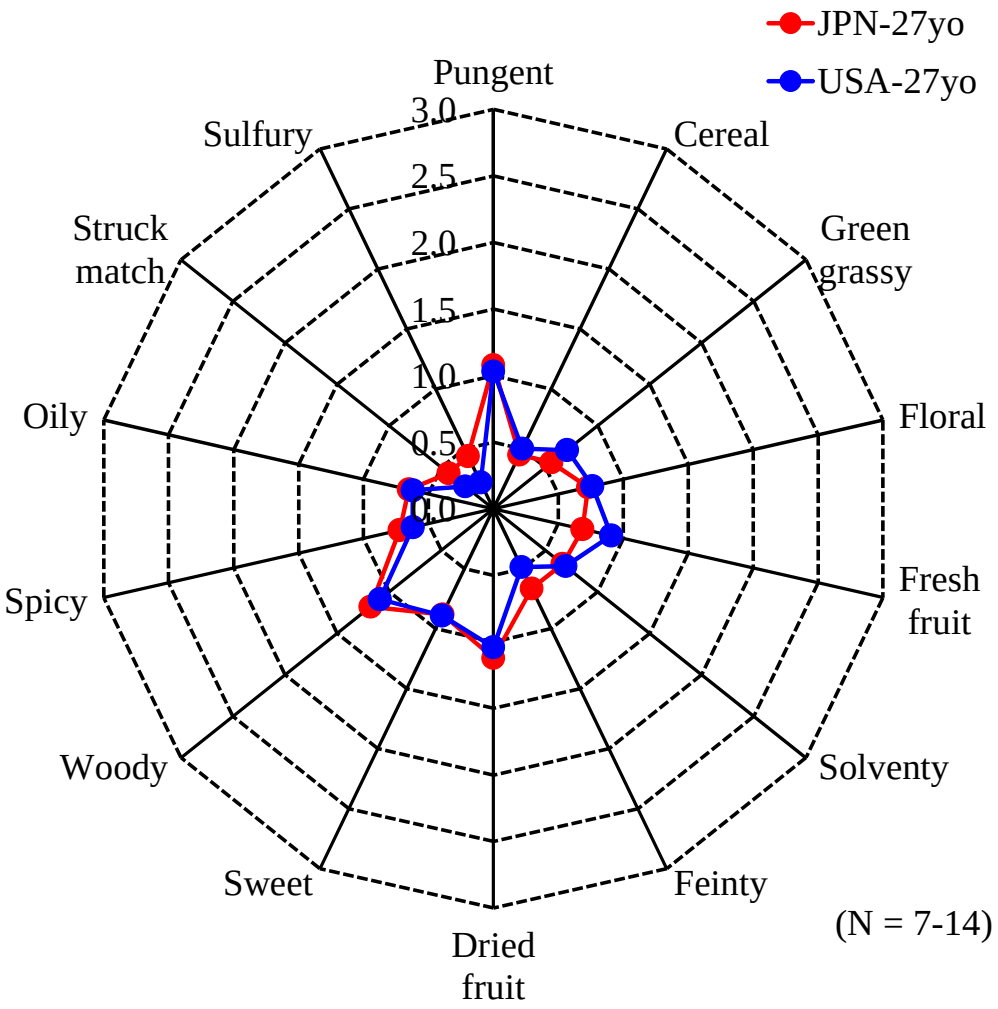


Figure 3.2 Spider diagram of sensory analysis results for 27 year old JPN and USA whiskies using standard aroma descriptors

The similarity in the patterns demonstrated in **Figure 3.2** suggest that this sensory evaluation did not accurately reflect the differences experienced when tasting the USA and JPN whiskies side by side. Therefore, it was suggested that a further in depth sensory analysis could be undertaken by developing new terminology which could be added to the whisky aroma wheel when working with Japanese whisky.

3.2.2 Chemical analysis of general volatile compounds and wood extractives

A range of JPN and USA whiskies (20, 27, and 40 years old) were subjected to chemical analysis for general volatile compounds and wood extractives which reflect some of descriptors in the aroma wheel (**Figure 3.1**). The focus of this study was on fatty acids (**Section 2.3.1**) which are associated with soapy and oily aromas, fatty acid esters (**Section 2.3.2**) linked with fruity and soapy aromas, as well as fusel alcohols (**Section 2.3.3**) described as feints and floral aromas and aromatics (**Section 2.3.4**) which contribute sweet and woody aromas (**Table 3.2-3**; Lee et al., 2001). These families of compounds were chosen because these compounds are quite general and common in whisky industry to compare whisky aromas and tastes.

A Spanish 20 year old grain whisky (SPN) was subjected to the same analysis, this was chosen because SPN whisky is reported to have a different type of aroma from USA whisky (Koshimizu, 2011) and JPN whiskies. In addition to this, grain whisky distilled by continuous still and purified more than pot still (**Section 1.1.1**) has fewer compounds of fatty acids and fatty acid esters in the new make spirit which makes it easier to compare the influence of the cask itself with other kinds of wood (Koshimizu, 2011).

Comparison of the 20 year old grain whiskies found that the JPN and SPN whiskies showed higher levels fatty acids, fatty acid esters, and aromatics than the USA whisky (**Table 3.2** and **Table 3.3**). Comparison between JPN and SPN whiskies found that they contained 14.1 mg/L and 11.5 mg/L respectively of total fatty acids, but in USA whisky this was lower at 1.0 mg/L (**Table 3.2**). Examination of the total fatty acid esters content determined that JPN and SPN whiskies contained 22.0 mg/L and 16.6 mg/L respectively, but in the USA whisky these compounds were found at a concentration of 5.3 mg/L

(**Table 3.2**). Analysis of the total aromatics established that JPN and SPN whisky contained 28.3 mg/L and 28.2 mg/L respectively, but in the USA whisky this was 16.4 mg/L (**Table 3.3**). In contrast to this the levels of total fusel alcohols were similar, around 1,100 mg/L in all whiskies analysed (**Table 3.2**).

When considering the 27 year old malt whiskies, the JPN whisky demonstrated higher levels all of fatty acids, fatty acid esters, fusel alcohols, and aromatics than USA whisky. The differences observed were not consistent and determined to be greater by a factor of 125%, 194%, 140%, and 170% respectively.

Analysis of the 40 year old malt whiskies determined that the JPN whisky was found to have higher levels of fusel alcohols and aromatics, but fewer fatty acids and fatty acid esters than were found in the USA whisky.

Fatty acids (except acetic acid) and fatty acid esters (except ethyl acetate) were only detected at very low levels (1.0, 11.5, 14.1 mg/L, and 5.3, 16.6, 22.0 mg/L respectively) in the 20 year old whiskies (**Figure 3.3**), but were detected at greater concentrations in the 27 and 40 year old samples (**Figure 3.3**). In contrast to this, the concentrations of acetic acid and ethyl acetate in the 20 year old whiskies were clearly detected (509.6, 618.9, 524.3 mg/L, and 662.7, 745.7, 587.5 mg/L respectively). Evaluation of the older 27 and 40 year old spirits found that when JPN whiskies were compared with USA whiskies, the concentrations of fatty acid esters are much higher in the 27 year old whisky (622.4 mg/L) than in the 40 year old whisky (285.8 mg/L).

Group	Flavour	Compound name	Concentration [mg/L 100% abv]						
			20yo			27yo		40yo	
			USA	SPN	JPN	USA	JPN	USA	JPN
Fatty acids	Soapy and oily	Acetic acid	509.6	618.9	524.3	891.1	798.9	773.2	1,404.9
		Propionic acid	N.D.	2.2	N.D.	1.9	2.5	1.8	7.0
		iso-Butyric acid	N.D.	N.D.	N.D.	1.7	1.6	0.6	1.9
		iso-Valeric acid	N.D.	N.D.	N.D.	3.6	4.7	1.4	4.0
		Hexanoic acid	N.D.	0.7	0.8	7.7	10.2	5.7	7.4
		Octanoic acid	N.D.	2.3	3.4	40.6	54.6	30.8	30.7
		Decanoic acid	1.0	4.1	6.3	67.6	82.8	64.7	63.3
		Dodecanoic acid	N.D.	2.3	3.7	45.1	53.9	39.8	34.8
(N = 1)	Total(-Acetic acid)		1.0	11.5	14.1	168.2	210.3	144.7	149.0
Fatty acid esters	Fruity and Soapy	Ethyl acetate	662.7	745.7	587.5	1,334.1	2,230.1	1,771.5	2,660.9
		Ethyl hexanoate	N.D.	N.D.	N.D.	13.5	29.9	12.9	15.2
		Ethyl octanoate	N.D.	3.6	4.8	71.9	153.1	70.7	62.0
		Ethyl decanoate	1.8	5.8	8.8	111.7	220.4	135.8	116.5
		Ethyl dodecanoate	1.5	4.5	6.4	76.2	138.0	86.9	64.6
		Ethyl tetradecanoate	N.D.	N.D.	N.D.	7.1	12.7	13.8	8.6
		Ethyl hexadecanoate	2.0	2.7	2.0	26.1	45.8	19.1	17.1
		Ethyl hexadecenoate	N.D.	N.D.	N.D.	14.2	22.4	2.3	1.8
(N = 1)	Total(-Ethyl acetate)		5.3	16.6	22.0	320.7	622.4	341.5	285.8
Fusel alcohols	Feints and floral	n-Propyl alcohol	405.3	410.0	408.0	408.6	526.0	455.8	495.7
		iso-Butanol	640.9	745.7	567.3	551.7	656.7	531.0	1,486.8
		iso-Amyl alcohol	104.1	52.2	55.0	1,231.7	1,823.8	1,728.7	3,472.8
		a-Amyl alcohol	29.5	12.7	15.5	346.3	556.6	374.8	929.9
		β-Phenyethyl alcohol	10.3	5.3	6.7	73.2	91.6	82.9	131.2
(N = 1)			1,190.1	1,225.8	1,052.5	2,611.5	3,654.7	3,173.1	6,516.4

Table 3.2 General volatile compounds in old whiskies

Group	Flavour	Compound name	Concentration [mg/L 100% as is]						
			20yo			27yo		40yo	
			USA	SPN	JPN	USA	JPN	USA	JPN
Aromatics	Sweet and woody	Vanillic acid	2.7	3.4	3.4	4.3	9.2	6.6	18.4
		Syringic acid	4.0	6.7	5.5	8.0	13.9	11.0	25.7
		Vanillin	2.6	5.5	6.0	4.1	7.9	7.2	18.4
		Syringaldehyde	6.5	11.6	12.1	12.4	18.3	16.7	34.8
		Sinapaldehyde	0.5	1.0	1.3	0.7	0.9	0.6	1.2
(N = 1)	Total		16.4	28.2	28.3	29.4	50.1	42.1	98.6

Table 3.3 Wood extractives in old whiskies

Greater variation was observed between concentrations of fatty acid esters than the fatty acids in the 27 and 40 year old whiskies. In the case of malt whiskies of 27 year and 40 year old, highest levels of fatty acid esters were in the 27 year old JPN whisky (622.4 mg/L) and lowest levels was found in the 40 year old JPN whisky (285.8 mg/L). The highest levels of fatty acids was 27 year old JPN whisky (210.3 mg/L) and lowest was 40 year old USA whisky (144.7 mg/L). This pattern was not found to be consistent between the whisky samples, although the highest levels of fatty acids (210.3 mg/L) and fatty acid esters (622.4 mg/L) were observed in the same 27 year old JPN whisky.

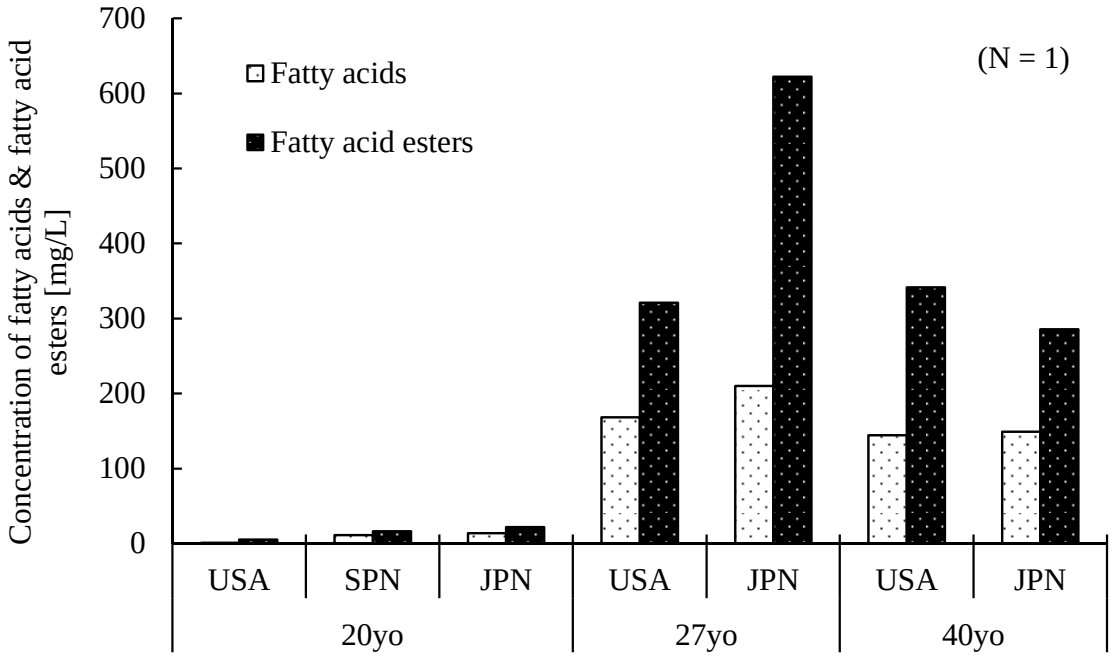


Figure 3.3 Fatty acids & fatty acid esters in old whiskies

Determination of the levels of fusel alcohols found that the 40 year old Japanese oak whisky had a much higher concentration of these compounds (6,516.4 mg/L) than all other whiskies (**Figure 3.4**). When the JPN whiskies were compared with USA whiskies and SPN whisky (20 year old), the concentrations of fusel alcohols were found to be in the range 1,052.5 to 1,225.8 mg/L, and did not show the previously observed

tendency to be higher than others, which was found to be the case for fatty acids and fatty acid esters. This observation was not reflected in the older whiskies, where the 27 and 40 year old whiskies were found to have between 2,611.5 and 6,516.4 mg/L of fusel alcohols. This pattern repeated that previously discerned in these samples for the concentration of fatty acid and fatty acid esters.

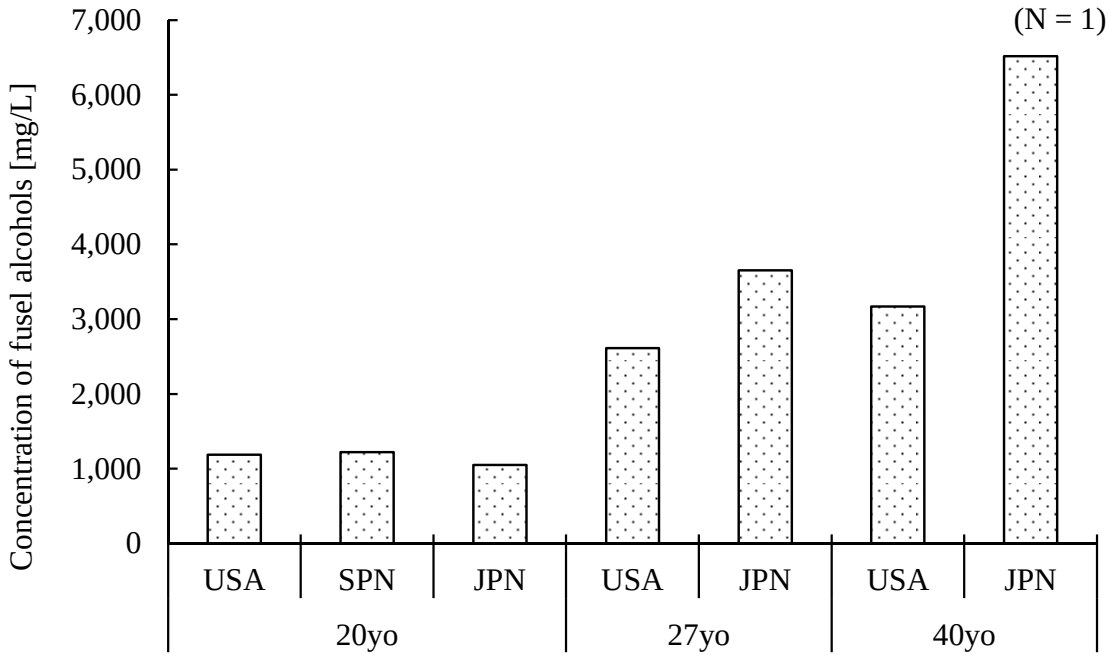


Figure 3.4 Fusel alcohols in old whiskies

Finally when considering the aromatic compounds (**Figure 3.5**), only the 40 year old JPN whisky demonstrated considerably higher concentration (98.6 mg/L) than found in all of other whiskies (concentrations ranged from 16.4 to 50.1 mg/L). The comparison of JPN and USA whiskies found that the concentrations of all the aromatics in JPN samples were greater than USA whiskies. However, when the 20 year old JPN whisky was compared with a 20 year old SPN whisky, these concentrations of aromatics were found to be similar. This suggests that JPN whisky contains more aromatics than USA whisky but this cannot

be said to only be characteristic of JPN whisky as SPN whisky also contains similar high levels of aromatic compounds.

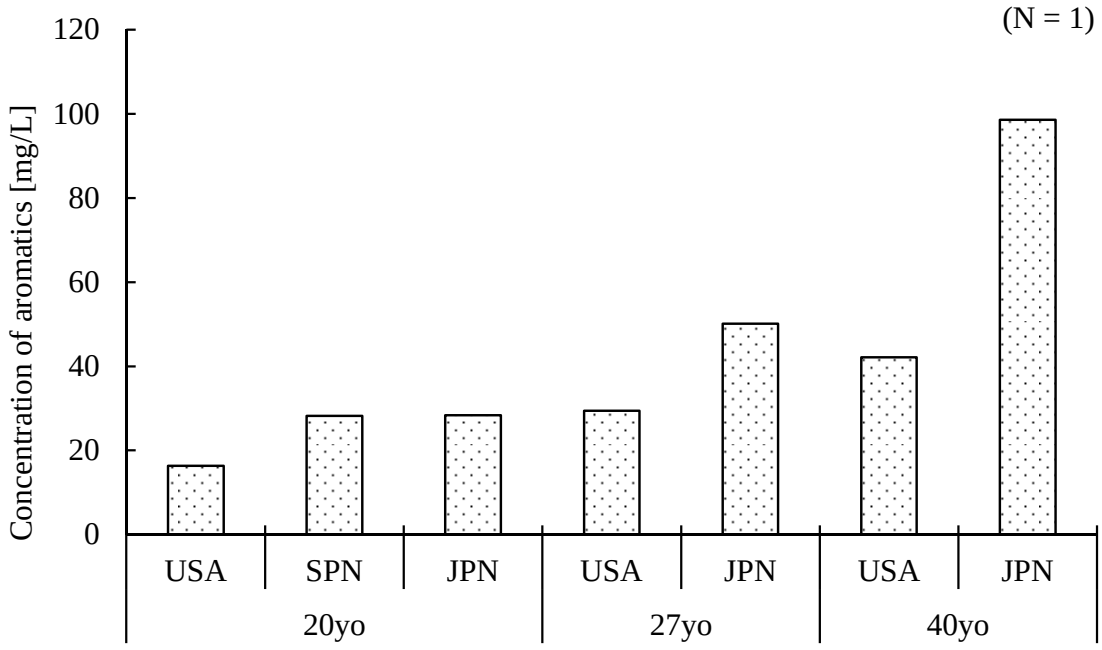


Figure 3.5 Aromatics in old whiskies

3.3 Discussion

This work began by determining if the Scotch whisky aroma wheel (**Figure 3.1**) was a suitable tool for the sensory assessment of Japanese whisky. The aroma wheel descriptors used in this study were found to not adequately express the differences between Japanese whiskies and those from the rest of the world. This was most noticeably demonstrated in the comparison of JPN and USA whisky (**Figure 3.2**). Following on from this discovery, work was carried out to look for suitable descriptors which could be used in addition to those used in the Scotch whisky industry. This would expand the terminology available and enable sensory scientists to describe whiskies in a

more global context. The aroma of JPN whisky had up to this point only ever been expressed using Japanese domestic descriptors. In order to look for a suitable descriptor which sensory panel can understand, new descriptor was needed meet this need.

Further identification of the uniqueness of JPN whisky was achieved by carrying out analytical methods common to the whisky industry.

Fatty acids with the exception of acetic acid and fatty acid esters (except ethyl acetate) were generally not detected in the 20 year old whiskies (**Figure 3.3**), but were detected in the 27 and 40 year old samples (**Figure 3.3**). It is suggested that the reason for this is that fatty acids and fatty acid esters are normally derived from the original new make spirit of malt whisky. The 27 year and 40 year whiskies were malt whiskies, but the only 20 year old whiskies were grain whiskies which are closer to neutral spirit with fewer aroma compounds due to the spirit distilled by continuous still and purified more than pot still (**Section 1.1.1**). Whereas, the concentrations of acetic acid and ethyl acetate were high because these compounds derived from not only new make spirit but also cask wood during maturation (Reazin, 1981).

When considering fatty acids of JPN and USA whiskies, the concentrations were mostly the same in all of three ages. When considering fatty acid esters, the concentration of 20 year old and 27 year old JPN whiskies were more than USA whiskies, however, the concentration of 40 year old JPN whisky was less than or similar to USA whisky. It is thought that this is probably due to the levels of these compounds found in the new make spirit and not derived from maturation. Therefore, it is suggested that the fatty acids and fatty acid esters levels are not a good indicator of the distinctive character of JPN whisky. In the case of fusel alcohols, only the 40 year old JPN whisky showed a higher

concentration compared to all of other whiskies. However, when JPN whiskies were compared with USA and SPN whisky for the 20 year and 27 year old samples, the concentrations were similar, and do not show the same tendency as observed in 40 year old whiskies. The reason why the higher concentration was found in the 40 year old JPN whisky is not known. It is suggested that this may be due to the original new make spirit and concentration occurring during very long periods of maturation (Conner et al., 2003). Considering the aromatics, only the 40 year old JPN whisky showed a much higher concentration. In addition to this the 20 year old JPN and SPN whiskies demonstrated more aromatics than USA whisky. The reason for the higher concentrations in 40 year old JPN whisky is not clear but this could be due to the different maturation conditions, such as temperature or humidity, experienced over very long periods of time.

When considering the 20 year old whiskies, JPN and SPN whiskies demonstrated increased concentrations of fatty acids and fatty acids esters than USA whisky. The reasons for this are unclear, it is postulated that the previous casks use (**Section 1.2**, Conner et al., 2003) may influence fatty acid and fatty acid ester content. A small quantity of residual whisky from the previous cask use fill may still be present at the point of refill, this may be absorbed into the wood or may still in the cask, this will combine with the next product as it matures (**Section 1.3**).

Therefore it is postulated that compounds derived from the new make spirit (fatty acids, fatty acid esters, and fusel alcohols) are not suitable indicators for defining the distinct character of JPN whisky. JPN whiskies demonstrated higher concentrations of aromatics than USA whiskies, but the concentration found in SPN whisky was also higher

than USA whisky and similar to JPN whisky. Therefore, aromatic compounds also are not indicative of the distinctive character of JPN whisky.

These results should not be considered to be surprising as new make spirit (and hence whisky) are all derived from cereals. Although there is some variation within the use of these cereals it may be difficult to use these compounds as defining characteristics of whisky matured in different casks. Therefore, in subsequent work, chemical analysis was used to focus on the woody aroma compounds which are more likely to be the source of the different aroma and aromas associated with JPN whisky.

Chapter 4: The identification of a unique character imparted by casks of Japanese oak

4.1 Introduction

The aroma of JPN whisky has previously been expressed using only Japanese domestic descriptors which represented challenges when sharing sensory data amongst international whisky researchers. This research began by looking for suitable descriptors, in addition to those which are generally used in whisky industry (**Section 3.1**). Descriptors are one of the most important tools to identify the unique character that differentiate whiskies. The aroma descriptors used are those frequently used by the whisky industry in both the UK and Japan, and which can be found on the Scotch whisky aroma wheel (**Figure 3.1**). As a result, the use of established whisky descriptors in this work did not adequately express the sensory experience of the JPN whisky under consideration (**Section 3.2.1**). The Scotch whisky aroma wheel, as suggested by the name was developed specially for 'Scotch' and therefore it is not comprehensive enough to describe the unique character of JPN whisky. Therefore, a further in depth sensory analysis was undertaken testing new terminology which could be added to whisky aroma wheel lexicon in order to develop a universal language which could be used with whiskies of global origin.

From the chemical analysis point of view, a previous lack of interest in whisky matured in JPN oak means that the typical chemical aroma compounds especially those which are characteristic of JPN oak, have yet to be identified. The volatiles compounds which are generally used across the whisky industry as measures of aroma consistency were chosen for the chemical analysis. In the previous chapter (**Section 3**), the fatty acids, fatty acid

esters, fusel alcohols, and aromatics were demonstrated to not be a good indicator of JPN whisky. In order to identify a specific compound which contributes to JPN whisky aroma, further chemical analysis would be required (**Section 3.2.2**).

Feedback from sensory panellists at SWRI was used to identify new words which could be used as descriptors for Japanese whisky aroma. The focus for the chemical analysis were the woody aroma compounds from which it was felt to be the most likely source of variation since the aromas of interest are linked with maturation in Japanese oak.

4.2 Results

4.2.1 Sensory analysis using special aroma descriptions

Sensory analysis was performed using the method and general descriptors described in **Section 3.1**. Panellists were also asked to comment on other aromas that they felt were present in the whiskies. Descriptions obtained from the panellists included the terms musty, earthy, varnish, incense, aniseed, matured pineapple, coconut, beeswax, and melted butter. From these comments, the descriptions of 'incense', 'matured pineapple', and 'coconut' which were the terms most frequently described by panellists and therefore were identified as possible aromas that typify JPN whiskies. The sensory analysis was repeated using these descriptions, in order to determine whether or not these attributes were recognised by the sensory panellists as characterising the unique aroma of JPN whisky.

Old whiskies which were felt to have a strong aroma, representative of typical JPN whiskies were compared the equivalent age of USA whisky using the aroma descriptions of incense, matured pineapple, and coconut. The summarised results are given for the 20 year old (**Table 4.1**), 27 year old (**Table 4.2**) and 40 year old (**Table 4.3**) whiskies.

		Incense	Matured pineapple	Coconut
JPN-20yo	Mean	1.4	0.8	1.4
	St. Dev.	0.7	0.6	0.7
	Minimum	0.3	0.1	0.6
	Maximum	2.0	1.8	2.5
USA-20yo	Mean	1.0	0.6	0.8
	St. Dev.	0.6	0.4	0.4
	Minimum	0.5	0.1	0.0
	Maximum	2.0	1.5	1.7
p value by t-test (N = 9)		0.40	0.26	0.09

Table 4.1 Sensory results of 20 year old JPN and USA whiskies using the aroma descriptions of incense, matured pineapple, and coconut

(Raw data is available in Appendix 1)

Examination of the 20 year old whiskies found that the intensities of incense and coconut were found to be scored equally with a mean score of 1.4 in JPN whisky, and they were similar 1.0 and 0.8 respectively in USA whisky. The highest intensity score for coconut was 2.5 in the JPN whisky. The differences between JPN and USA whisky in these three descriptors were not found to be significant ($p = 0.40, 0.26, 0.09$ respectively).

When considering the 27 year old whiskies, the intensities of incense and coconut were found to be different in JPN whisky and these mean scores were 1.8 and 0.9 respectively. The score of the incense aroma was greater than that of coconut aroma, whereas the scores for 20 year old JPN whisky were same 1.4 (**Table 4.1**). In the 27 year old USA whisky, these characteristics were also found to be different (1.1 and 0.5 respectively). However, these scores were all lower than those for the JPN whisky.

In both the 27 year old JPN and USA whiskies the incense character was found to be the most intense and the coconut character the least intense. This did not reflect the results when analysing the 20 year old JPN and USA whiskies. It should be noted that some panellists did not pick up the matured pineapple and coconut character in USA whisky (observed in the minimum scores found in **Table 4.2**). Analysis of the results in **Table 4.2** determined that the scores for JPN and USA whiskies in 'incense' and 'coconut' were significantly different ($p = 0.01$ and 0.05 respectively).

		Incense	Matured pineapple	Coconut
JPN-27yo	Mean	1.8	1.3	0.9
	St. Dev.	0.8	0.8	0.6
	Minimum	0.5	0.1	0.2
	Maximum	2.9	2.5	2.5
USA-27yo	Mean	1.1	1.0	0.5
	St. Dev.	0.7	0.7	0.3
	Minimum	0.5	0.0	0.0
	Maximum	2.7	2.5	1.0
p value by t-test (N = 9)		0.01	0.22	0.05

Table 4.2 Sensory results of 27 year old JPN and USA whiskies using the aroma descriptions of incense, matured pineapple, and coconut

(Raw data is available in Appendix 2)

Analysis of the 40 year old whiskies found that the intensities of incense and coconut were similar (2.1 and 1.8 respectively) in JPN whisky, but they were different and much lower 1.1 and 0.6 in USA whisky. The intensity of matured pineapple was lowest 1.0 in JPN whisky, but had a mid-range score in the USA whisky. Some panellists did not pick up the coconut character in USA whisky. The intensity of matured pineapple was widely spread from 0.0 to 3.0 and its standard deviation was high 1.0. It is suggested that this variation in scores may be due to panellists not understanding the properties of this attribute or there may be a wide range of individual aroma thresholds for this attribute. The differences between JPN and USA whiskies in incense and coconut attributes were founded to be significant ($p = 0.02$ and 0.01 respectively).

		Incense	Matured pineapple	Coconut
JPN-40yo	Mean	2.1	1.0	1.8
	St. Dev.	0.7	1.0	1.0
	Minimum	0.8	0.0	0.5
	Maximum	3.0	3.0	3.0
USA-40yo	Mean	1.1	0.8	0.6
	St. Dev.	0.6	0.7	0.3
	Minimum	0.5	0.1	0.0
	Maximum	2.3	2.0	1.0
p value by t-test (N = 9)		0.02	0.52	0.01

Table 4.3 Sensory results of 40 year old JPN and USA whiskies using the aroma descriptions of incense, matured pineapple, and coconut

(Raw data is available in Appendix 3)

The mean values for these characteristics were plotted for incense (**Figure 4.1**), matured pineapple (**Figure 4.2**) and coconut (**Figure 4.3**). In the case of the incense descriptor (**Figure 4.1**), JPN whiskies at all ages of maturation were scored to have higher intensities than the USA whiskies. In the USA whiskies the score was similar across all ages, whereas in JPN whiskies the score increased with increasing age of the spirit (or length of maturation). These differences between JPN and USA whiskies became greater in older age spirits, and they were significantly different in 27 year old ($p = 0.01$) and 40 year old ($p = 0.02$) whiskies.

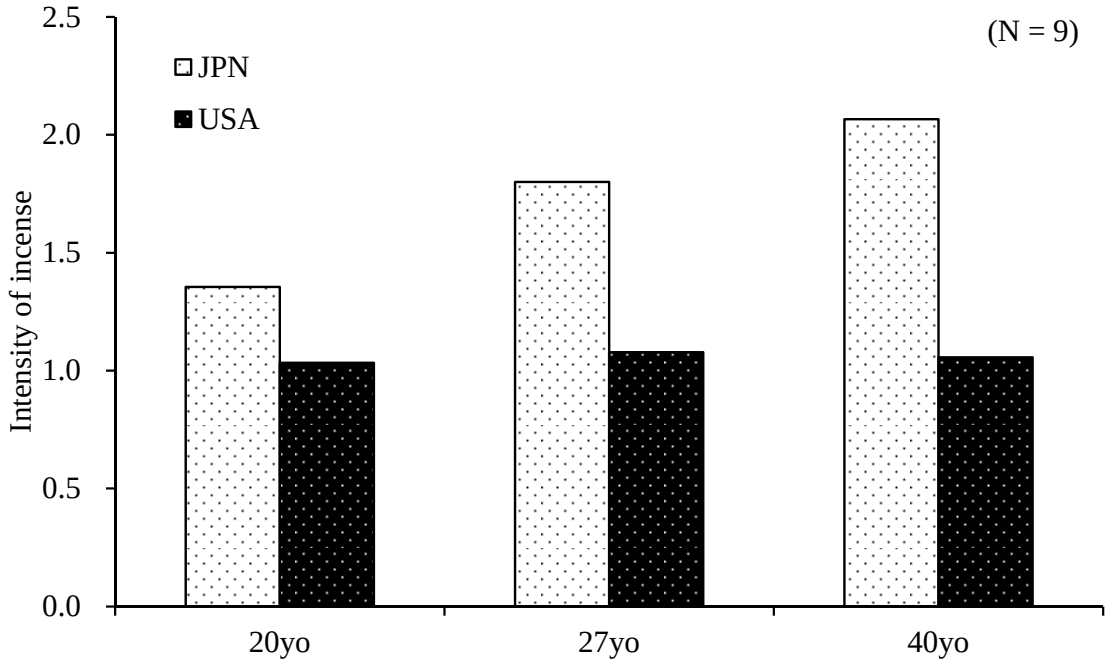


Figure 4.1 Sensory results of old whiskies for 'incense' aroma descriptor

In the case of matured pineapple descriptor (**Figure 4.2**) JPN whiskies for all ages were determined to have higher intensities. However, these differences were not significant for any of the ages examined ($p = 0.26, 0.22, 0.52$). When considering the JPN whiskies, the matured pineapple character was considered to be greatest in the 27 year old spirit. This was also the case for the 27 year old USA whisky. The lowest across for this descriptor when considering geography was interestingly both the 20 year old products, JPN and USA whiskies.

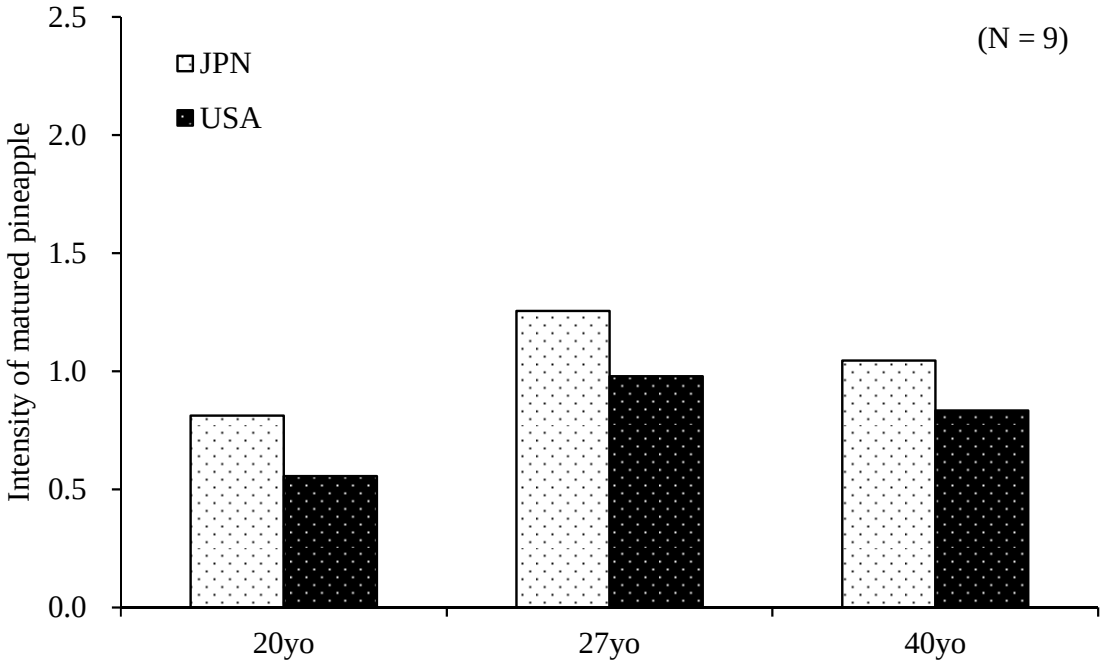


Figure 4.2 Sensory results of old whiskies for 'matured pineapple' aroma descriptor

In the case of the coconut descriptor (**Figure 4.3**), again JPN whiskies in all ages were scored to have higher intensities than USA whiskies. These differences were determined to be significant in 27 year old ($p = 0.05$) and 40 year old ($p = 0.01$) whiskies.

When considering the USA whiskies this coconut character appeared to decrease over time. In the JPN whiskies this was not the case and the 27 year old demonstrated a lower score for coconut compared to the 20 year old. In contrast the 40 year old was observed to have a much higher score.

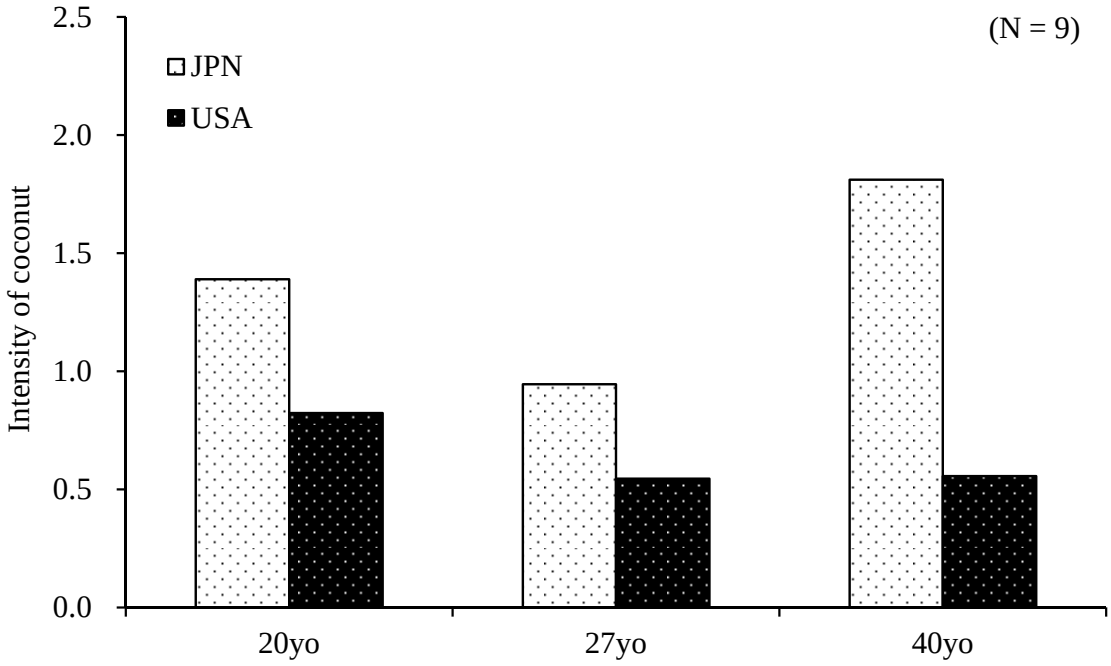


Figure 4.3 Sensory results of old whiskies for 'coconut' aroma descriptor

As described above, it was determined that the JPN whiskies clearly had higher scores for the incense and coconut attributes compared to the USA whiskies at both 27 years old and 40 years old. These differences were statistically significant when comparing the older products.

4.2.2 Chemical analysis of whisky lactone

In **Section 4.2.1**, the coconut descriptor was determined to be one of the typical aromas of JPN whisky. Although coconut was determined to be present in USA whisky, this was lower than in JPN whisky and also declined over increased maturation, the inverse of what was observed in JPN whisky. Whisky lactone is a wood extractive known to be responsible for coconut aroma in whisky (Otuska et al., 1974). This molecule contains two chiral carbons and as such can exist as four isomers, 3S,4S (*cis*) (**Figure 4.4**), 3S,4R (*trans*) (**Figure 4.5**), 3R,4R (*cis*), and 3R,4S (*trans*). Although each isomer has a

slightly different aroma, they all have coconut notes and could potentially contribute to the overall coconut aroma in whisky (Koppenhoefer et al., 1994). Masson et al. (1995) reported that only two of these isomers are reported to exist naturally, 3S,4S (*cis*), 3S,4R (*trans*). Another research group reported that the isomerisation of these lactones will not occur in wine or whisky because of the acidic nature of these media (Pollnitz et al., 1999). Therefore it can be said that the conformation of whisky lactone is not affected by the conditions of maturation.

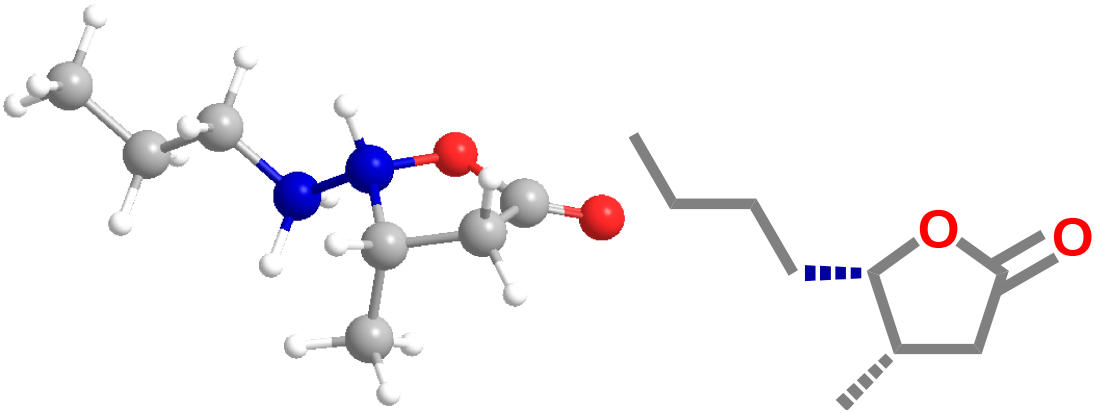


Figure 4.4 Chemical structure of 3S,4S *cis*-whisky lactone

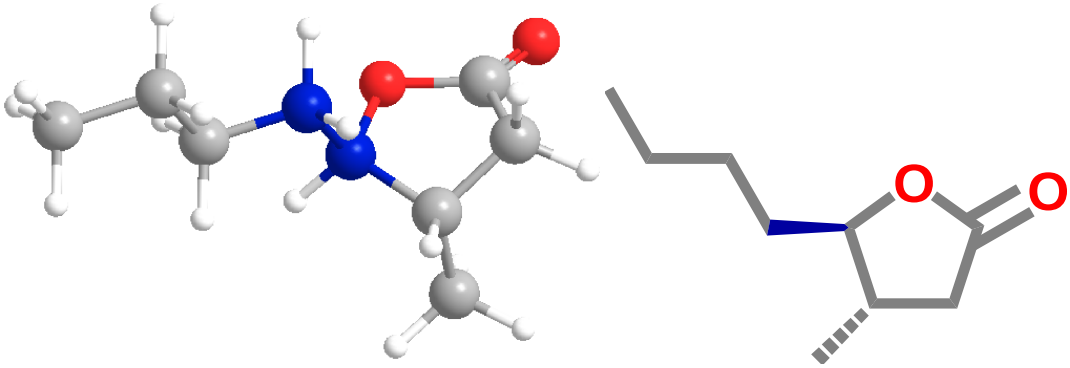


Figure 4.5 Chemical structure of 3S,4R *trans*-whisky lactone

Chemical analysis of lactones in old whiskies was carried out using GC-MS according to the method in **Section 2.3.5**. The whiskies used in this study were 20 year old grain

whiskies from Japanese, American, and Spanish oak, and malt whiskies from Japanese and American oak (27 and 40 year old).

Whisky (N = 1)		*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
20yo	USA	3.02	0.30	3.32	10.24
	SPN	2.08	0.28	2.36	7.56
	JPN	1.22	2.47	3.69	0.49
27yo	USA	2.72	0.47	3.19	5.79
	JPN	2.27	2.52	4.78	0.90
40yo	USA	6.37	1.54	7.91	4.14
	JPN	14.78	29.60	44.38	0.50

Table 4.4 Chemical analysis results of whisky lactones in old whiskies

In the USA and JPN 20 year old whiskies, the total amount of lactone was found to be similar (3.32 mg/L and 3.69 mg/L respectively), whereas in the SPN whisky the level was lower (2.36 mg/L) (**Table 4.4**). When comparing the 27 year old whiskies, USA whisky was determined to have similar level (3.19 mg/L) to the 20 year old (3.32 mg/L). In contrast, the concentrations in the JPN 27 year old whiskies were greater (4.78 mg/L) compared to that of 20 year old. When comparing the 40 year old whiskies, USA whisky was greater (7.91 mg/L) compared to that of 27 year old whisky (3.19 mg/L), but JPN whisky was much greater (44.38 mg/L) compared to that of 27 year old whisky (4.78 mg/L).

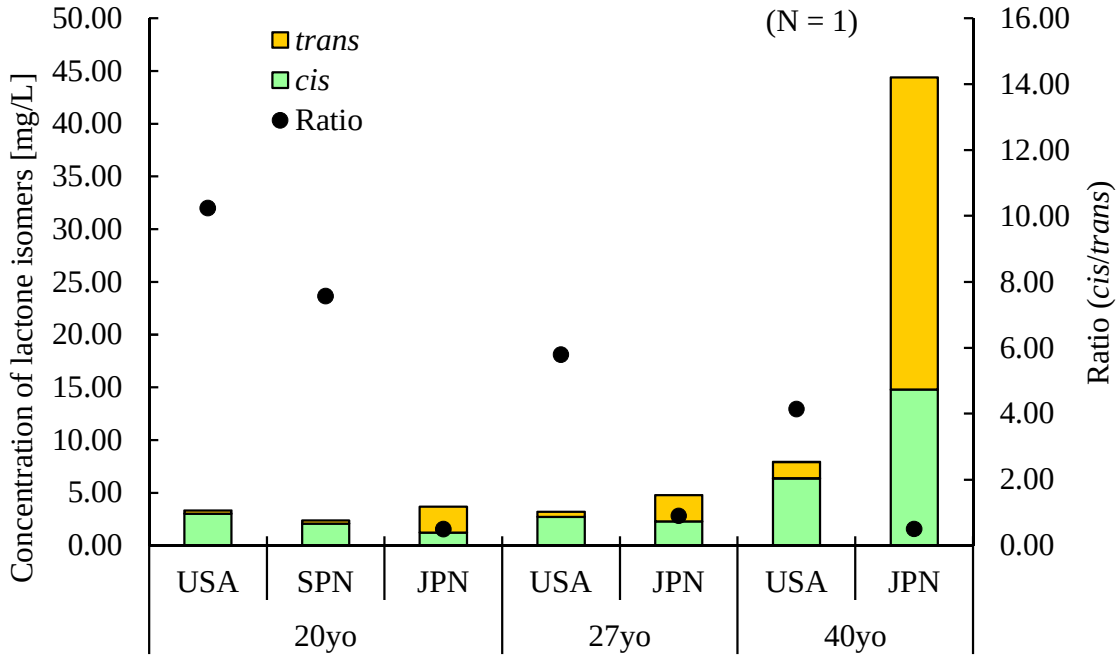


Figure 4.6 Chemical analysis results of whisky lactones in old whiskies

All of ages of JPN whiskies were found to contain higher levels of *trans*-lactone (2.47 mg/L, 2.52 mg/L, 29.60 mg/L) than USA whiskies (0.30 mg/L, 0.47 mg/L, 1.54 mg/L) (**Table 4.4** and **Figure 4.6**). The ratio of *cis/trans* in all of the JPN whiskies were all less than 1.0 (0.49, 0.90 and 0.50) while in the SPN and USA whiskies the ratios were all well in excess of 1.0 (10.24, 7.56, 5.79, 4.14). Hence, only in the JPN whiskies were the levels of *trans*-lactone higher than *cis*-lactone. These differences are clearly illustrated in **Figure 4.6**.

These observations are important because previous studies by Nabeta et al. (1986) reported that the levels of lactones differed among three types of oak, including *Q. mongolica*. The *Q. mongolica* showed higher levels of *trans*-lactone than the other types of oak considered by these authors (**Figure 4.7**). The wood of *Q. mongolica* is also known as Japanese oak which was used in the construction of casks for the maturation of the JPN whisky. Therefore this study confirms these previous observation

by Nabeta et al. (1986), although sadly these authors did not consider American (*Q. Alba*) or European (*Q. robur* or *Q. petraea*) oaks in their work. The results from **Section 4.2.2** led to the proposal of the hypothesis that casks constructed of *Q. mongolica* produces whiskies which contain higher ratios of *trans*-lactones than casks constructed of American or European oak.

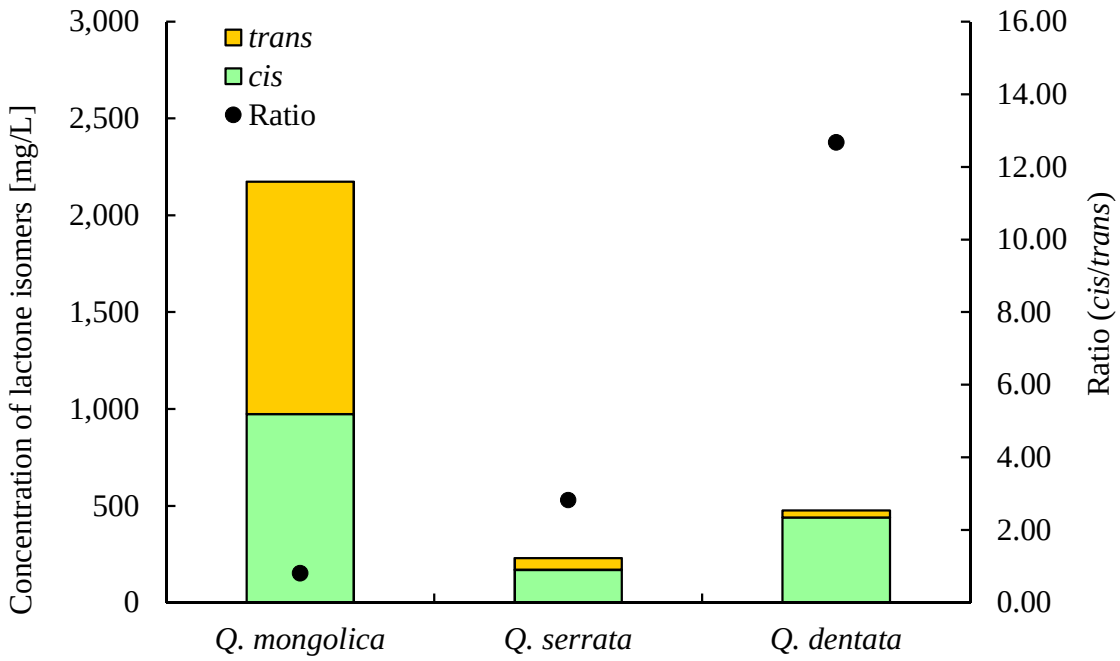


Figure 4.7 Whisky lactone amounts in wood reported by Nabeta et al. (1986)

In order to test this hypothesis, wood chips were collected from the whole surface (to a depth of approximately 2 mm) of casks that had been used for the extended maturation of old JPN and USA whiskies. The details of these old whiskies are outlined in **Section 2.1.1** (**Table 2.1)**. Since these casks could not be disassembled, as these were not exhausted casks at the end of their life, these chips were collected from the outside surface of casks. The extraction using these chips with 60% abv spirit for one day at room temperature was carried out according to the method outlined in **Section 2.2.1**. The

concentration of lactones was determined by GC-MS following to the method in **Section 2.3.5**.

The results are presented as the lactone concentration and the ratio of lactone isomers and are illustrated in **Figure 4.8** with further detail given in **Table 4.5**. When considering 20 year old USA and JPN whiskies, the total amount of lactone in USA (13.82 mg/L) was slightly higher than JPN (9.78 mg/L). In contrast, when comparing 27 year old whiskies, USA (9.01 mg/L) was found to be lower than JPN (22.19 mg/L). In the case of 40 year old whiskies, USA (10.12 mg/L) were slightly lower than JPN (13.14 mg/L). Comparison of the *trans*-lactone content of the 20, 27, and 40 year old whiskies, all ages of JPN (6.55mg/L, 11.90 mg/L, 8.43 mg/L) showed higher level than USA (1.24 mg/L, 1.14 mg/L, 0.99 mg/L). When comparing the ratio, all ages of JPN (0.49, 0.86, 0.56) had a ratio of less than 1.00, but in contrast the ratio demonstrated by USA whiskies (10.18, 6.90, 9.22) were all greatly in excess of 1.00. This reflects the results presented earlier in this chapter (**Figure 4.6**) which also found that the ratios of *cis/trans* in all of the JPN whiskies were all were less than 1.0 while in the USA whiskies the ratios were all well in excess of 1.0.

Cask (N = 1)		*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* +*trans*) [mg/L as is]	Ratio (*cis* /*trans*)
20yo	USA	12.58	1.24	13.82	10.18
	JPN	3.23	6.55	9.78	0.49
27yo	USA	7.87	1.14	9.01	6.90
	JPN	10.29	11.90	22.19	0.86
40yo	USA	9.13	0.99	10.12	9.22
	JPN	4.72	8.43	13.14	0.56

Table 4.5 Whisky lactone amounts in wood collected from the cask surface of old whiskies by the extraction using 60% abv solution

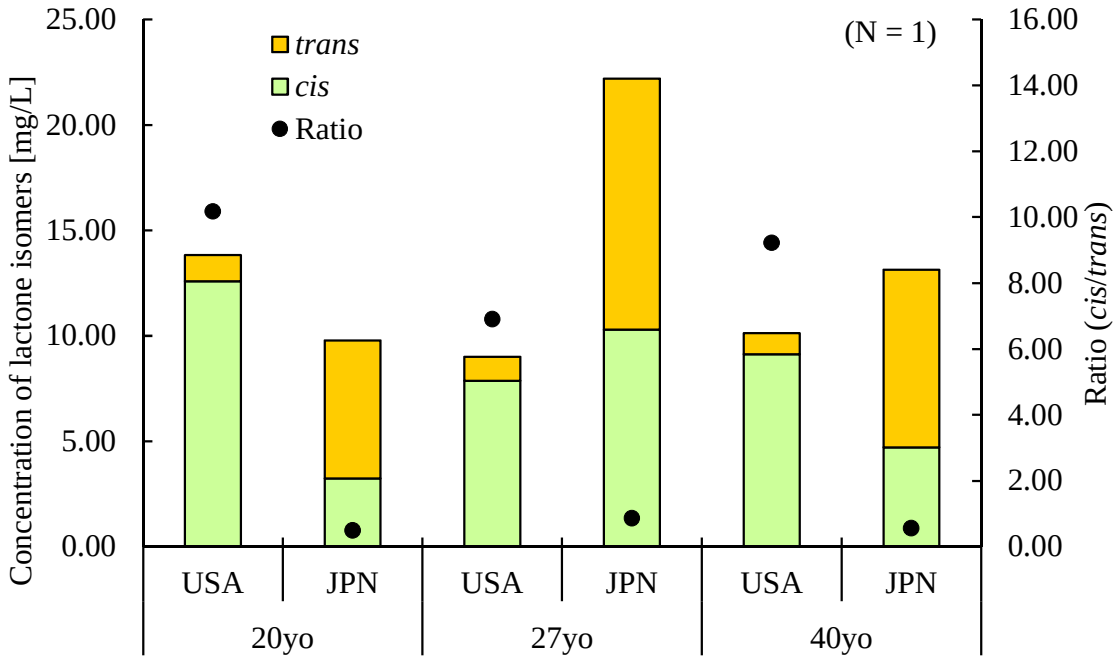


Figure 4.8 Whisky lactone amounts in wood collected from the cask surface of old whiskies by the extraction using 60% abv solution

4.3 Discussion

The aim of this work was to determine suitable specific descriptors for JPN whiskies, which are not part of the current language used to describe Scotch whisky. Analysis of sensory panellists comments were used to determine new language, and three descriptors 'incense', 'matured pineapple', and 'coconut' were concluded to be unique to Japanese whisky. Repeated sensory analysis using these three descriptors found that the JPN whiskies clearly had higher scores for incense and coconut attributes compared to the USA whiskies (**Table 4.1-3**). These two descriptors, incense and coconut are common and useful ones for communication between European and Japanese whisky researchers, and are felt to be good expressions of the unique character of JPN whiskies.

Following on from the above work, chemical analysis focussed on the coconut aroma compound, which is known to be 'whisky lactone'. It was found that JPN whiskies have different ratios of lactone isomers, weighted towards greater relative levels of *trans*-lactone than was observed in USA whiskies (**Table 4.4**). This has not previously been reported in the literature surrounding this area of whisky science. In the case of the 40 year old whisky, JPN whisky showed much higher concentration of both lactones. The reason for this is unknown, but can be assumed that whisky maturation is normally influenced by not only the cask extraction but also storage conditions in the warehouse (**Section 1.1.2**). Therefore, 40 year old whiskies are thought to have had a greater influence from the conditions in the surrounding environment during maturation and therefore these differences were greater.

Further to this, when the ratio was determined in the extraction of lactones from wood chips at laboratory scale and the ratios between American and Japanese oak, Japanese

oak showed higher relative levels of *trans*-lactone (**Table 4.5**). The total amounts extracted from each wood type were not consistent, but the pattern observed within the ratios were similar between casks of USA whisky and JPN whisky. Therefore, due to the similar ratios of lactone found between laboratory wood chip extract experiments and those found in whisky suggests that the analysis of wood chip extracts is a suitable model to rapidly mimic a long period cask maturation. These results support the hypothesis that the relatively high levels of *trans*-lactone in JPN whiskies are very likely to be derived from the wood itself.

Many previous studies on the ratio of *cis*- and *trans*-lactone isomers have been reported in the wine industry (Waterhouse and Towey, 1994; Diaz-Plaza et al., 2002; Simon et al., 2003). From these reports the reported ratios for *cis*/*trans* lactone isomers in American oak, were between 7.21 and 10.20. For wine matured in European oak wood, the ratio ranged from 1.40 to 4.79. Analysis of the data presented in these papers demonstrates that in these oaks the *cis*-lactone is the more abundant isomer. Therefore it is postulated that only Japanese oak has higher ratio of *trans*-lactone than European or American oaks. Therefore, it is suggested that the *cis/trans* lactone ratio may provide a means of identifying by analytical methods whether or not a whisky has been matured in Japanese oak. This is useful information for selection of cask staves which will give strong coconut aroma. These results may also be useful when looking to confirm the authenticity of Japanese whiskies in the market place as demand for these products increases.

Chapter 5: The behaviour of whisky lactone isomers in Japanese oak casks

5.1 Introduction

In **Section 4**, it was determined that JPN whisky has a unique coconut aroma which could be identified by a professional sensory panel. Whisky lactone is a wood extractive known to be responsible for coconut aroma in whisky (Otuska et al., 1974), and it was reported that only two of isomers exist naturally although four isomers exist due to two chiral carbons contained in the molecule (Masson et al., 1995). Following further investigation, the ratio of whisky lactone isomers was found to be different in Japanese oak to European and American oaks (**Section 4.2.2**). Here, the factors (**Section 1.1**, **Section 1.2**) that influence the amount of lactone, and the ratio of lactones in matured spirit were studied in order to determine the key factors involved in aroma activation.

Whisky maturation is long process involving complicated reactions (**Section 1.1.2**), and is still yet to be fully understood. Therefore, a simple experiment using cask wood chip extractions in an ethanol solution was developed. This could then be used to simulate whisky maturation in a reduced period of time. Additionally, the potential of heat treatment was also studied to determine if toasting or charring influenced lactone content and ratio in spirit. The rationale was that heat treatment is known to be a good method to regenerate casks (**Section 1.3**), when casks are regenerated by re-charring, thermal degradation of lignin yields aroma compounds similar to those produced in a new charred cask (Conner et al., 2014).

Whisky casks are stored for many years in bonded, secure warehouses without moving or rolling, often with little environmental control. Whisky in casks naturally evaporates at the rate of 2 or 3% annually in Japan (Koshimizu, 2011), known in Scotland as 'the angels' share', and this causes the volume of liquid in the cask (**Figure 5.1**) to decrease (Conner et al., 2014). It therefore is suggested that it would be reasonable to assume that the top stave would become dry over time as it has reduced contact with the liquid, the bottom stave would be wet, with some staves in the top third of the cask having variable contact as evaporation takes place over many years. It was postulated that the extraction of wood component especially lactone, depends upon the position of the stave in the cask.

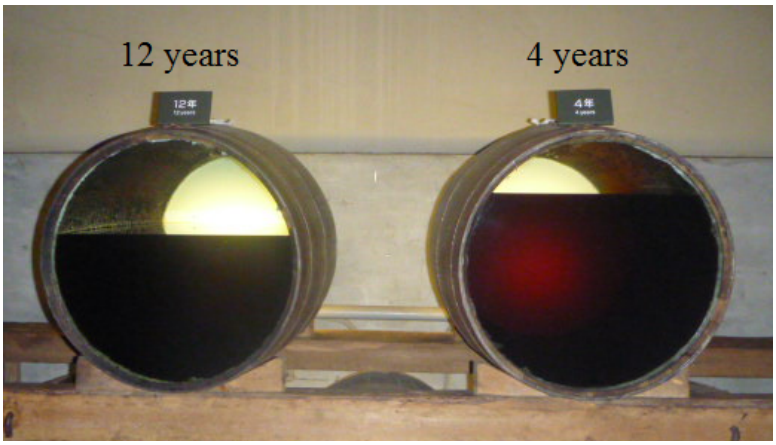


Figure 5.1 Model casks of whisky evaporation at Yamazaki Distillery

5.2 Results

5.2.1 Wood extracts analysis

The ratio of lactones in the extracted 60% abv ethanol solution for one day was analysed and was found to be similar to the ratios in matured whisky (**Section 4.2.2**). From this

result, it was suggested that wood chips extract using an ethanol solution for a short period of one day can imitate the extraction of lactones into spirit occurring within the cask. However, the method outlined in **Section 2.2.1** was a technique in development and required further validation. In the work presented here the methodology previously described was further developed, focussing on the length of extraction and spirit strength. In addition to this, the influence of stave position and extraction behaviour at different depth of the stave during maturation on lactones was studied using the method developed in this chapter. The wood chips required for this work were collected from the within about two mm of the outside surface of a Japanese oak cask. The specific cask was selected from inventory information, and allowed a cask to be selected which was similar to those in which the JPN 27 year old whisky (**Table 2.1** (**Section 2.1.1**)) was matured in the previous study.

5.2.1.1 Extraction period

In order to establish whether one day (**Section 2.2**) was an adequate period of time for extraction, additional periods of 2, 7, and 30 days extraction were performed using Japanese oak chips by stirring with 60% abv ethanol solution at room temperature (approximately 20°C). The lactone concentration and the ratio of lactone isomers are presented in **Table 5.1** and illustrated in **Figure 5.2** which demonstrates the cumulative concentration of lactones. The *cis*-lactone concentrations of all extraction lengths ranged from 5.07 to 5.44 mg/L which were similar. The *trans*-lactone concentrations of all extraction lengths ranged from 4.96 to 5.35 mg/L, which were also very similar. The *cis*/*trans* ratios for all length of time were from 1.02 to 1.03 which were within a close range. Statistical analysis determined that the p values were greater than 0.05 (**Section 2.5**), and therefore no significant difference (**Table 5.1**) in either isomer was shown under

the conditions examined. From this work it could be concluded that a period of one day is sufficient for the extraction of whisky lactones for analysis.

		cis lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
1 day	Mean	5.44	5.35	10.80	1.02
	St. Dev.	0.36	0.32	0.68	0.01
2 days	Mean	5.07	4.96	10.03	1.02
	St. Dev.	0.09	0.14	0.20	0.03
7 days	Mean	5.30	5.13	10.43	1.03
	St. Dev.	0.09	0.13	0.21	0.02
30 days	Mean	5.35	5.21	10.56	1.03
	St. Dev.	0.09	0.11	0.20	0.01
p value by ANOVA (N = 4)		0.18	0.15	0.15	0.70

Table 5.1 Effect of extraction length of wood chip on subsequent extraction of lactones into 60% abv solution

(Raw data is available in Appendix 4)

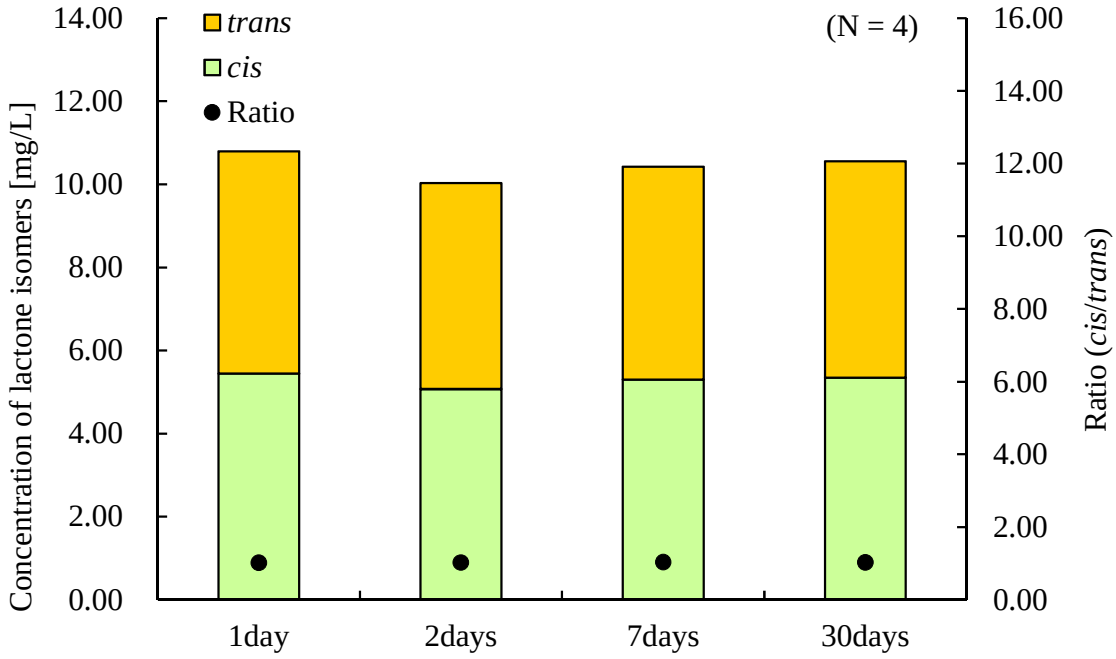


Figure 5.2 Effect of extraction length of wood chip on subsequent extraction of lactones into 60% abv solution

5.2.1.2 Extraction strength

The influence of spirit strength as alcohol by volume on lactone extraction and the ratio of these isomers was examined. In addition to a concentration similar to that of whisky cask strength (60% abv) (**Section 1.1.1**), extractions using dilutions of 20% abv, 40% abv, 80% abv were also carried out using Japanese oak chips. The spirit/wood chip mixture was stirred for one day at room temperature (approximately 20°C). The levels of *cis*- and *trans*- isomers were evaluated (**Table 5.2**), and the cumulative data presented in **Figure 5.3**. The concentration of *cis*-lactone 20% abv experiment was found to be 3.35 mg/L, however the concentrations of other strengths ranged from 5.42 to 5.79 mg/L. The concentration in the *trans*-lactone of 20% abv dilution was found to be 3.33 mg/L, whereas the concentrations of other strengths ranged between 5.20 to 5.72 mg/L. The *cis*/*trans* ratio for all spirit strengths were found to be between 1.01 and 1.04 which were

similar values. Statistical analysis found that the p value of the ratio was greater than 0.05 (**Section 2.5**), meaning that no significant differences in the isomer ratio was detected (**Table 5.2**). However, concentrations of both isomers were significantly different (p = <0.01). The lower extract levels found in the in the 20% abv solution agreed with the previous observations on the hydrophobicity of whisky lactones published by Atanasova et al. (2004).

		cis lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* +*trans*) [mg/L as is]	Ratio (*cis* /*trans*)
20% abv	Mean	3.35	3.33	6.67	1.01
	St. Dev.	0.30	0.30	0.58	0.04
40% abv	Mean	5.42	5.20	10.62	1.04
	St. Dev.	0.59	0.57	1.16	0.01
60% abv	Mean	5.44	5.35	10.80	1.02
	St. Dev.	0.36	0.32	0.68	0.01
80% abv	Mean	5.79	5.72	11.51	1.01
	St. Dev.	0.16	0.15	0.31	0.01
p value by ANOVA (N = 4)		<0.01	<0.01	<0.01	0.28

Table 5.2 Effect of extraction alcohol strength of wood chip on subsequent extraction of lactones into ethanol solution

(Raw data is available in Appendix 5)

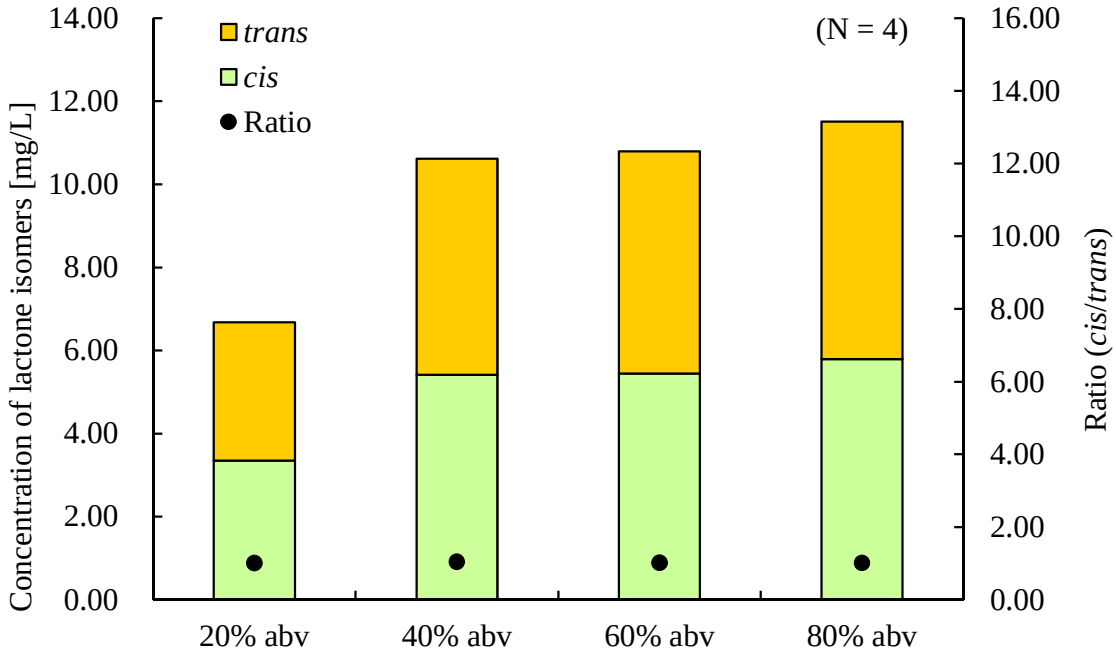


Figure 5.3 Effect of extraction alcohol strength of wood chip on subsequent extraction of lactones into ethanol solution

5.2.1.3 Stave position

The hypothesis that the position of the stave in the cask would influence the extraction of wood components was tested using the extraction experimental protocol. Wood chips were collected from within approximately 2 mm outside surface of the staves which had previously been in positions representing the top, middle, and bottom of the casks (**Section 1.2**). Experiments were also performed using chips which represented a mixture of all the stave positions in the cask. The chips used in this experiment were collected from casks used to mature the whiskies outlined in **Table 2.1** (**Section 2.1.1**). The extraction using these chips was carried out by stirring with 60% abv spirit for one day at room temperature (**Section 2.2**). The results of concentration and ratio of lactone isomers are shown in **Table 5.3**.

Cask (N = 1)		Position	*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
JPN	20yo	Top	5.07	7.49	12.56	0.68
		Middle	5.20	7.23	12.43	0.72
		Bottom	2.86	3.72	6.58	0.77
		All	3.23	6.55	9.78	0.49
	27yo	Top	12.71	11.35	24.06	1.12
		Middle	10.04	18.23	28.27	0.55
		Bottom	8.28	15.60	23.88	0.53
		All	10.29	11.90	22.19	0.86
	40yo	Top	3.42	13.93	17.35	0.25
		Middle	5.22	4.36	9.57	1.20
		Bottom	6.74	20.61	27.35	0.33
		All	4.72	8.43	13.14	0.56
USA	20yo	Top	9.32	0.75	10.07	12.43
		Middle	8.13	0.98	9.11	8.30
		Bottom	11.81	1.17	12.98	10.09
		All	12.58	1.24	13.82	10.18
	27yo	Top	4.52	0.69	5.21	6.55
		Middle	12.66	0.86	13.52	14.72
		Bottom	11.09	0.78	11.87	14.22
		All	7.87	1.14	9.01	6.90
	40yo	Top	15.23	1.34	16.57	11.37
		Middle	8.88	0.91	9.79	9.76
		Bottom	6.95	0.73	7.68	9.52
		All	9.13	0.99	10.12	9.22

Table 5.3 Effect of stave position of wood chip on subsequent extraction of lactones into 60% abv solution

The data from **Table 5.3** was plotted to directly compare the extractions of 20 year old (**Figure 5.4**), 27 year old (**Figure 5.5**) and 40 year old (**Figure 5.6**) cask staves.

When the 20 year old JPN whisky cask was considered, the total amount of lactones from the 'top' and 'middle' stave samples were found to be similar at, 12.56 and 12.43 mg/L, the 'bottom' stave sample was lower at 6.58 mg/L. The one containing 'all' stave samples was 9.78 mg/L which was in between the concentrations of 'top' and 'middle', and 'bottom' staves. Comparison of the ratios of sample 'top', 'middle', and 'bottom' were similar 0.68, 0.72, 0.77, however the value for the 'all' sample was 0.49 not as expected to give a value in the middle of the other experiments. In the case of the 20 year old USA whisky, the total amount of lactones in all positions were close 10.07, 9.11, 12.98 mg/L and the mixture known as 'all' was also similar at 13.82 mg/L. The ratios of all positions were within a similar range 12.43, 8.30, 10.09, and 'all' was also close 10.18.

Examination of the 27 year old JPN whisky wood determined that, the total amount of lactone in all positions were close in concentration 24.06, 28.27, 23.88 mg/L and 'all' was also similar 22.19 mg/L. This was in contrast to the ratio of lactones where the 'top' was 1.12, but the 'middle' and 'bottom' were 0.55 and 0.53 respectively. The 'all' sample had a ratio of 0.86 which was between 'middle' and 'bottom' ratios. In the case of 27 year old USA whisky, the total amount of 'top' was 5.21 mg/L, but 'middle' and 'bottom' were close 13.52 and 11.87 mg/L. The one of 'all' was 9.01 mg/L which was between 'top', and 'middle' and 'bottom' ratios.

In the case of 40 year old JPN whisky, the total amounts of each position demonstrated greater variability, 17.35, 9.57, 27.35 mg/L. In the case of 40 year old USA whisky, the

total amounts of each position were also variable at 16.57, 9.79, 7.68 mg/L respectively, but the extent of variability was smaller than observed with the 40 year old JPN whisky.

When JPN whiskies were compared with USA whiskies and the total amount of lactones were found to be similar when considering the 20 year old whiskies (**Figure 5.4**). However, in 27 year old samples, the levels of lactone in JPN whisky were greater than USA whisky (**Figure 5.5**). In 40 year old, the ones of both JPN whisky and USA whisky were variable and no pattern could be discerned (**Figure 5.6**).

All ages of JPN whiskies demonstrate higher levels of *trans*-lactones than the USA whiskies confirming the pattern observed in **Section 4.2.2**, thus adding further weight to the confirmation of that hypothesis. When focussing the analysis on the ratio (**Table 5.3**), the ratio of JPN whisky appeared consistent, between 0.25 and 1.20. The ratios of USA whisky were more variable than JPN whisky, but all of the ratio were greater than 6.00. It is suggested that the amount of *trans*-lactone in this wood was small compared with those of *cis*-lactone. However, when considering the total amount of lactones (**Figure 5.4-6**), these were found to be inconsistent and did not show the same tendency as was observed with the ratio values. The reasons for these differences remain unclear, but may be related to the original habitat of the oak tree, or age of the tree at the time of felling.

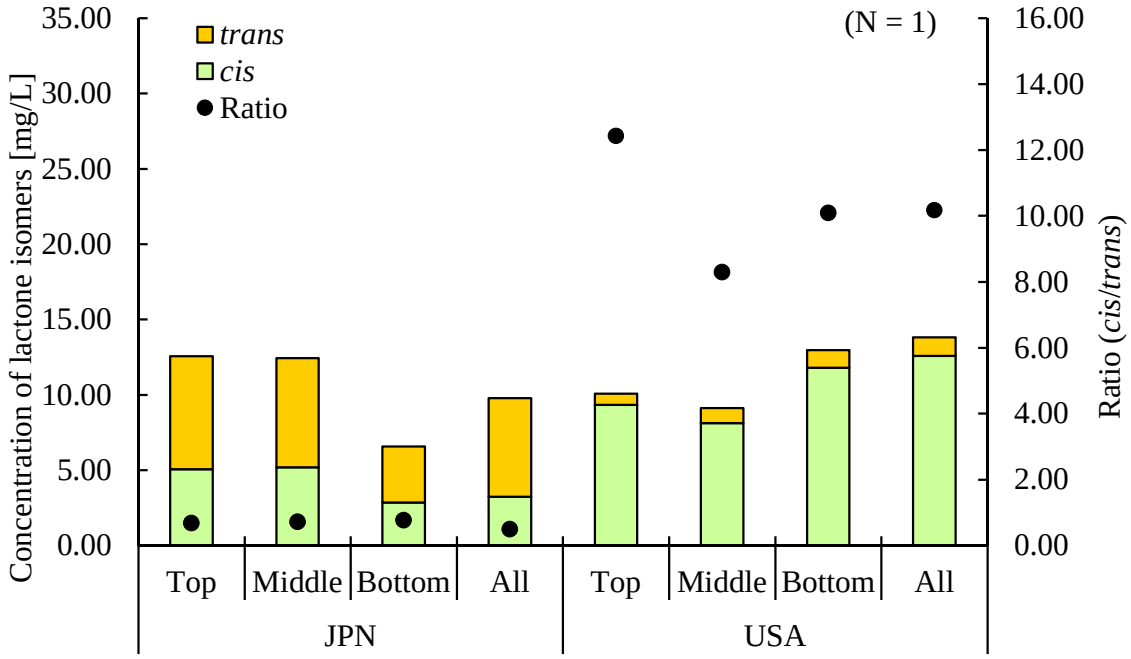


Figure 5.4 Effect of stave position of wood chip for casks of 20yo whiskies on subsequent extraction of lactones into 60% abv solution

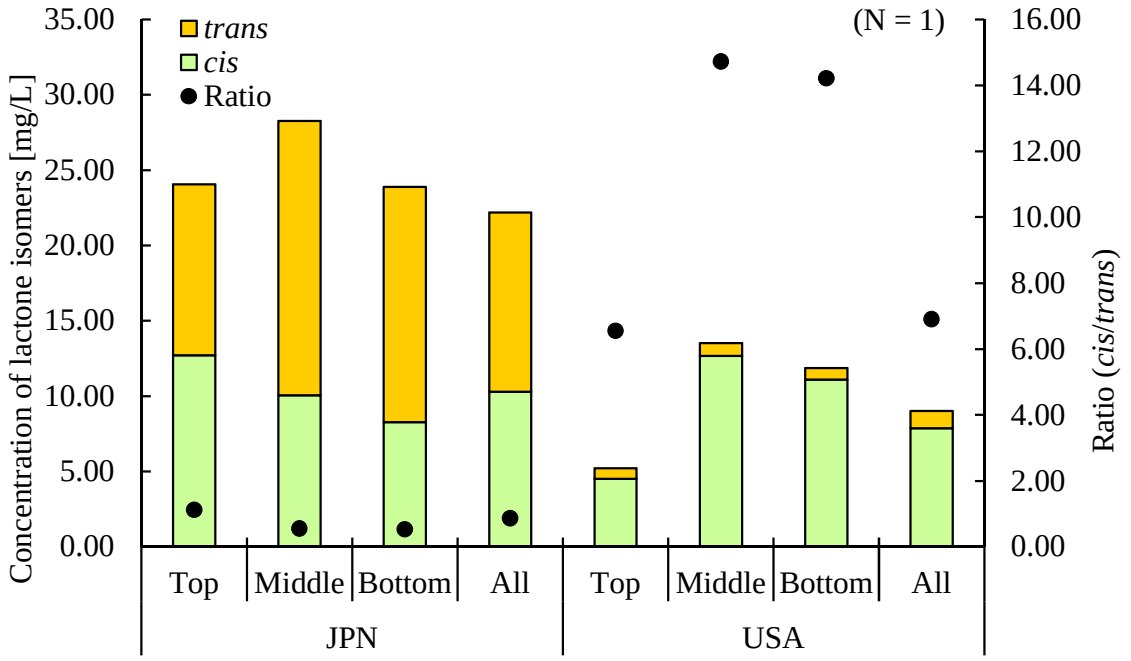


Figure 5.5 Effect of stave position of wood chip for casks of 27yo whiskies on subsequent extraction of lactones into 60% abv solution

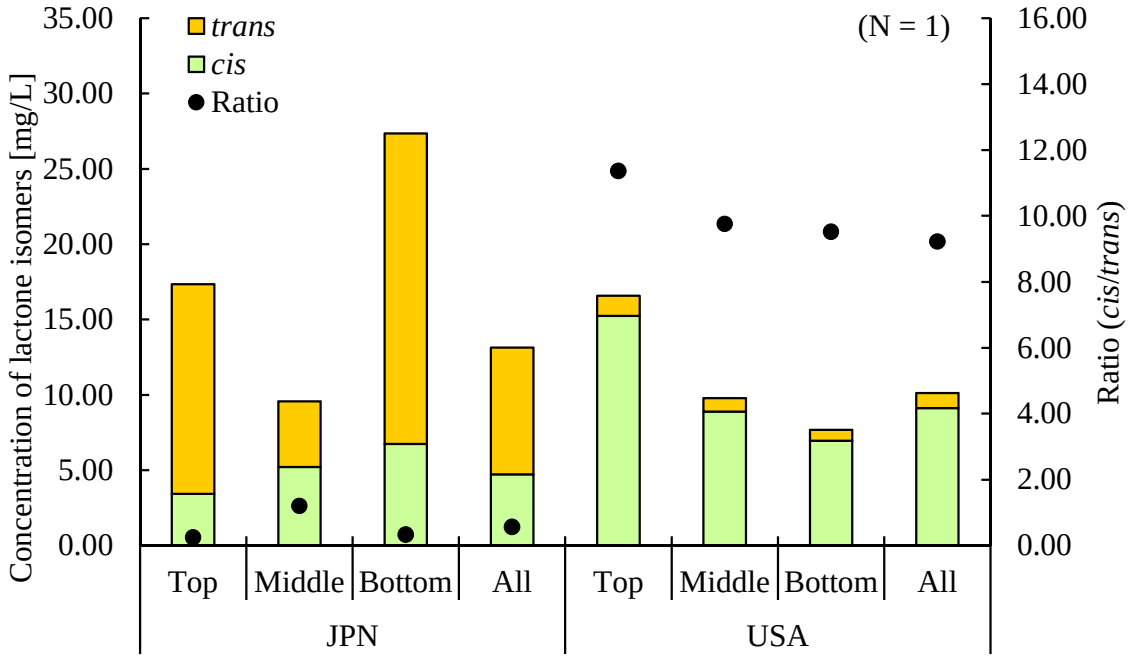


Figure 5.6 Effect of stave position of wood chip for casks of 40yo whiskies on subsequent extraction of lactones into 60% abv solution

5.2.1.4 Different depths within the stave of Japanese oak

The work presented earlier in this chapter (**Section 5.2.1.3**) regarding stave position yielded inconsistent results and the reasons for this were unclear. In order to explore the impact of the stave on spirit, the influence of depth of spirit penetration was investigated. A stave from the bottom of a Japanese oak cask which was selected from inventory information and had been used in whisky maturation for more than 30 years is presented in **Figure 5.7**. The depth of visual whisky soaking into this stave was around 9 mm (by visual analysis) from the internal surface.

Figure 5.7 Visual whisky soaking into the stave

A series of one millimetre slices of wood were taken from different depths of the stave pictured in **Figure 5.7**. These were collected using a wood slicer according to the method in **Section 2.1.2** and the extraction using these chips was carried out according to the previous used extraction methods of 60% abv ethanol solution for one day (**Section 2.2.1**). The resultant samples of spirit were analysed for lactone content (**Section 2.3.5**), colour (**Section 2.2.4**), and aromatic compounds (**Section 2.3.4**). The data for lactone concentration is presented in **Table 5.4** and colour and aromatic compounds in **Table 5.5**. The cumulative data are respectively presented in **Figure 5.8** and **Figure 5.9**.

The concentration of *cis*-lactone was 0.48 mg/L at a depth of 1 mm, but this decreased to 0.25 mg/L by a depth of 5 mm. From 6 mm depth, this increased to 1.85 mg/L at the 34 mm although this increasing was not constant at some depths, 14, 17, 19, 24 mm. The concentration of *trans*-lactone was 0.78 mg/L at 1 mm which was greater than *cis*-lactone at 1 mm. This also once decreased but increased again and kept increasing with sudden decreases at some depths reflecting the pattern previously observed with the *cis*-lactone levels. Comparison of the *cis*/*trans* ratio determined that the values were similar, ranging between 0.49 and 0.65, because both lactones demonstrated similar trend.

Depth (N = 1) [mm]	*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* +*trans*) [mg/L as is]	Ratio (*cis* /*trans*)
1	0.48	0.78	1.26	0.61
2	0.31	0.52	0.82	0.60
3	0.25	0.40	0.65	0.63
4	0.27	0.55	0.82	0.49
5	0.25	0.38	0.63	0.65
6	0.29	0.51	0.80	0.56
7	0.35	0.63	0.97	0.55
8	0.36	0.58	0.94	0.62
9	0.40	0.74	1.14	0.54
10	0.44	0.81	1.24	0.54
11	0.47	0.82	1.29	0.57
12	0.66	1.31	1.98	0.51
13	0.61	1.21	1.82	0.51
14	0.57	1.02	1.59	0.56
15	0.76	1.46	2.22	0.52
16	0.79	1.54	2.33	0.51
17	0.62	1.14	1.76	0.54
18	0.83	1.58	2.42	0.53
19	0.76	1.47	2.22	0.52
20	0.74	1.38	2.12	0.53
21	1.16	2.19	3.35	0.53
22	1.13	2.17	3.29	0.52
23	1.11	2.13	3.25	0.52
24	0.89	1.68	2.57	0.53
25	1.10	2.09	3.19	0.53
26	1.18	2.24	3.43	0.53
27	1.27	2.42	3.69	0.53
34	1.85	3.61	5.46	0.51

Table 5.4 Lactone extraction from different depths within the Japanese oak stave using 60% abv solution

The colour (**Table 5.5**) was 0.36 ABS at 1 mm depth, peaking at 1.05 ABS at 9 to 11 mm. After the peak the colour level decreased. The data for colour stop at 21 mm, because further samples were lost before the analysis.

In the case of vanillic acid, the concentration at the depth of 1 mm was high 0.43 mg/L, but following this (2 mm) dropped suddenly down to 0.24 mg/L. At a deeper depth between 3 and 9 mm, these concentrations gradually increased and then maintained around same level of 1.20 mg/L, although the levels demonstrated some variability between 1.17 and 1.54 mg/L.

When vanillin was examined, the concentrations found at the depth of 1 mm was high at 0.48 mg/L, but the value at 2 mm was suddenly down reflecting the same pattern as observed with vanillic acid. At deeper depths between 3 and 13 mm, these concentrations gradually increased. Between 13 and 19 mm the concentration kept same level around 0.70 mg/L before gradually increasing again.

The concentrations of syringic acid were found to be high at a depth of 1 mm (0.67 mg/L), but at 2 mm was decreased which was the same as pattern as observed for vanillic acid and vanillin. At deeper depths the levels of syringic acid increased, reaching 2.59 mg/L at 27 mm.

The concentration of syringaldehyde at 1 mm was high which was same as previously observed in vanillin and syringic acid. From 2 mm depth, this concentration was gradually increased up to 1.84 mg/L at 26 mm.

The concentrations of sinapaldehyde at the 1 mm and 2 mm were almost same around 1.00 mg/L. That was gradually increased to 0.98 mg/L at 17 mm and kept same level of around 1.00 mg/L.

Depth (N = 1) [mm]	Colour [ABS]	Vanillic acid [mg/L as is]	Vanillin [mg/L as is]	Syringic acid [mg/L as is]	Syringaldehyde [mg/L as is]	Sinapaldehyde [mg/L as is]
1	0.36	0.43	0.48	0.67	1.24	0.14
2	0.40	0.24	0.26	0.40	0.80	0.11
3	0.45	0.38	0.21	0.32	0.71	0.15
4	0.55	0.59	0.21	0.28	0.75	0.19
5	0.67	0.60	0.22	0.30	0.73	0.21
6	0.82	0.74	0.28	0.24	0.72	0.23
7	0.94	0.92	0.35	0.26	0.74	0.29
8	1.03	0.95	0.38	0.27	0.77	0.36
9	1.05	1.19	0.44	0.28	0.78	0.44
10	1.05	1.24	0.52	0.31	0.70	0.53
11	1.05	1.17	0.57	0.35	0.83	0.59
12	1.02	1.34	0.61	0.45	0.92	0.67
13	0.98	1.36	0.67	0.53	1.02	0.77
14	0.87	1.17	0.67	0.62	1.05	0.79
15	0.87	1.34	0.71	0.78	1.20	0.87
16	0.82	1.21	0.70	1.00	1.22	0.92
17	0.82	1.37	0.72	1.16	1.36	0.98
18	0.82	1.35	0.72	1.33	1.46	1.00
19	0.83	1.47	0.73	1.47	1.53	0.98
20	0.85	1.42	0.81	1.68	1.64	1.03
21		1.54	0.98	1.83	1.73	0.97
22		1.50	0.97	1.96	1.80	1.04
23		1.31	0.99	2.02	1.74	0.99
24		1.38	1.10	2.26	1.84	1.04
25		1.33	1.16	2.37	1.84	0.98
26		1.35	1.23	2.58	1.84	0.99
27		1.17	1.30	2.59	1.79	0.98
34		1.52	1.68	2.03	1.27	1.21

Table 5.5 Colour & aromatics extraction from different depths within the Japanese oak stave using 60% abv solution

This result showed that the colour was most intense in chips relating to stave depths of 9 - 11 mm, with the colour at 8 and 12 mm also very similar. This pattern was not observed for lactones and other aromatics. Only vanillin was determined to show a sudden increase concentration at 21 mm, this might be similar extraction kinetics as observed for colour. However, the other aromatics were extracted consistently with no apparent relationship with depth to which whisky had previously soaked into the stave. No change was observed in the lactones ratio (**Table 5.4**).

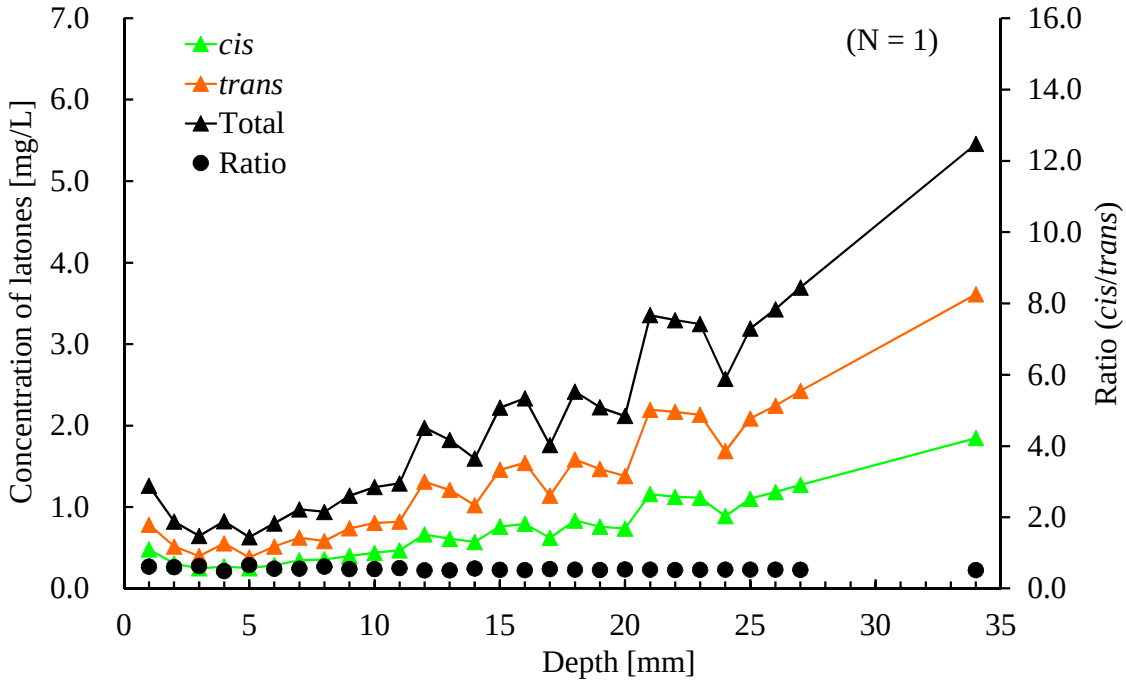


Figure 5.8 Lactones from different depths within the Japanese oak stave using 60% abv solution

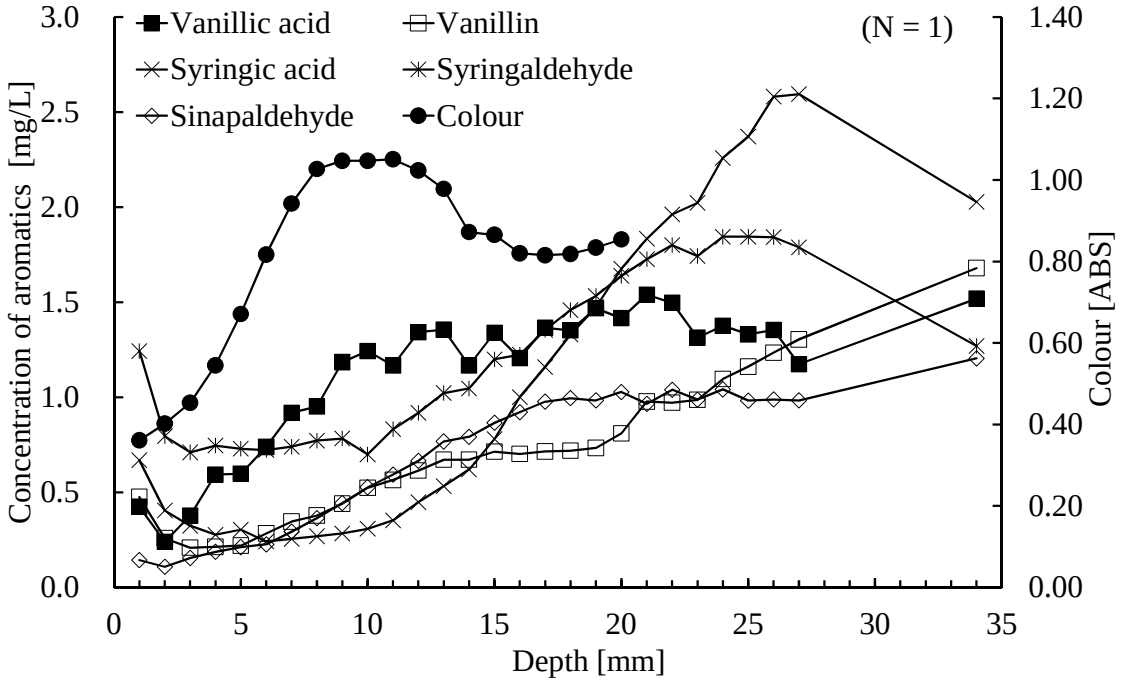


Figure 5.9 Colours and aromatics from different depths within the Japanese oak stave using 60% abv solution

5.2.2 Effect of heating conditions

Whisky casks are often treated using heat and this has benefit of regenerating the inner surface of the cask when it was exhausted (**Section 1.3**; Conner et al., 2014). When a cask is described as exhausted this means distillers would not expect the cask to be capable of a further contribution of the woody aroma compounds (**Section 1.1.2.1**; Nishimura et al., 1989). In the following work, the potential of cask regeneration by heat treatment in order to increase the lactone extraction into the maturing spirit was examined. Stave slices were heat treated under different conditions and the lactone content of spirit determined following mixing with 60% abv ethanol solution for one day (**Section 2.2.1**).

5.2.2.1 Heat treatment of wood chips

The effects of heating conditions were studied by using Japanese and American oak chips which were collected from 'all' positions of staves from casks (defined in **Section 5.2.1.3**) which were selected from inventory information and had been used for whisky maturation for more than 30 years. Wood chips were heat treated using gas chromatography oven according to the method in **Section 2.2.2**. Extractions using 20% abv ethanol solution for one day were performed using the method in **Section 2.2.1** and lactones measured using GC-MS (**Section 2.3.5**).

The data collected following heat treatment of chips are presented in **Table 5.6**. For both Japanese and American oak chips, under the higher heat conditions, 250°C for more than four minutes and 300°C for more than two minutes, the total amount of lactone was clearly less than 250°C for less than two minutes and lower than 210°C. It is suggested that these lactones were degraded during the treatment due to the high temperature. When both types of chips were treated under the milder conditions and were compared, at the JPN whisky, following treatment at 180°C was found to have the lowest concentration of *cis* and *trans*-lactone, 1.43 and 2.68 mg/L respectively. At a temperature of 210°C for a duration of four minutes the highest levels of *cis* and *trans*-lactone (3.87 and 5.63 mg/L) were observed. Although these two conditions produced a large difference in the concentration of lactone extracted, the ratio was similar (0.53 and 0.69). In the case of USA whisky, the concentration of *cis*-lactone ranged from 3.70 to 4.44 mg/L and *trans*-lactone were from 0.21 to 0.42 mg/L, although all the ratios were variable, these were all greater than 10.

	Temp. [°C]	Time [min]	*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
JPN (N = 1)	No treatment		3.18	5.01	8.19	0.64
	130	2	3.11	4.62	7.72	0.67
	150	2	2.25	3.31	5.56	0.68
	180	2	1.43	2.68	4.11	0.53
	210	2	2.71	4.13	6.83	0.66
		4	3.87	5.63	9.50	0.69
		6	3.05	4.31	7.36	0.71
	250	2	2.28	3.55	5.82	0.64
		4	1.20	2.29	3.49	0.52
		6	1.48	2.49	3.97	0.60
	300	2	1.31	1.59	2.90	0.82
		4	1.39	1.83	3.22	0.76
		6	N.D.	N.D.		
USA (N = 1)	No treatment		4.40	0.42	4.83	10.36
	130	2	4.32	0.33	4.65	13.20
	150	2	3.88	0.27	4.15	14.13
	180	2	3.70	0.21	3.91	17.44
	210	2	4.44	0.33	4.77	13.50
		4	4.33	0.38	4.71	11.42
		6	3.91	0.34	4.25	11.68
	250	2	4.05	0.25	4.30	16.06
		4	3.13	0.09	3.22	34.57
		6	2.82	0.05	2.88	51.51
	300	2	0.92	N.D.		
		4	0.55	N.D.		
		6	0.72	N.D.		

Table 5.6 Effect of different heat treatments of stave slices on subsequent extraction of lactones into 60% abv solution

The results of the conditions of 250°C for less than two minutes and the milder temperature and time conditions are illustrated in **Figure 5.10**. It is suggested that under the harsher conditions the wood lactones were degraded (**Table 5.6**) and therefore these results were not included in the data analysis. Under all of the conditions illustrated, the ratios of lactones from Japanese oak were consistent ranging from 0.53 up to 0.71. The ratios of lactones extracted from American oak were higher, with a wider range from 10.36 to 17.44. It is suggested that this may be due to the very low amounts of *trans*-lactone compared to *cis*-lactone (**Figure 5.10**). This was a similar result to that previously demonstrated in **Section 5.2.1.3**, thus further strengthening these observations. The total amounts of lactone from Japanese oak ranged from 4.11 to 9.50 mg/L and a consistent pattern in these concentrations could not be detected. The amounts of lactones from American oak varied from 3.91 to 4.83 mg/L and demonstrated consistency across the conditions illustrated in **Figure 5.10**.

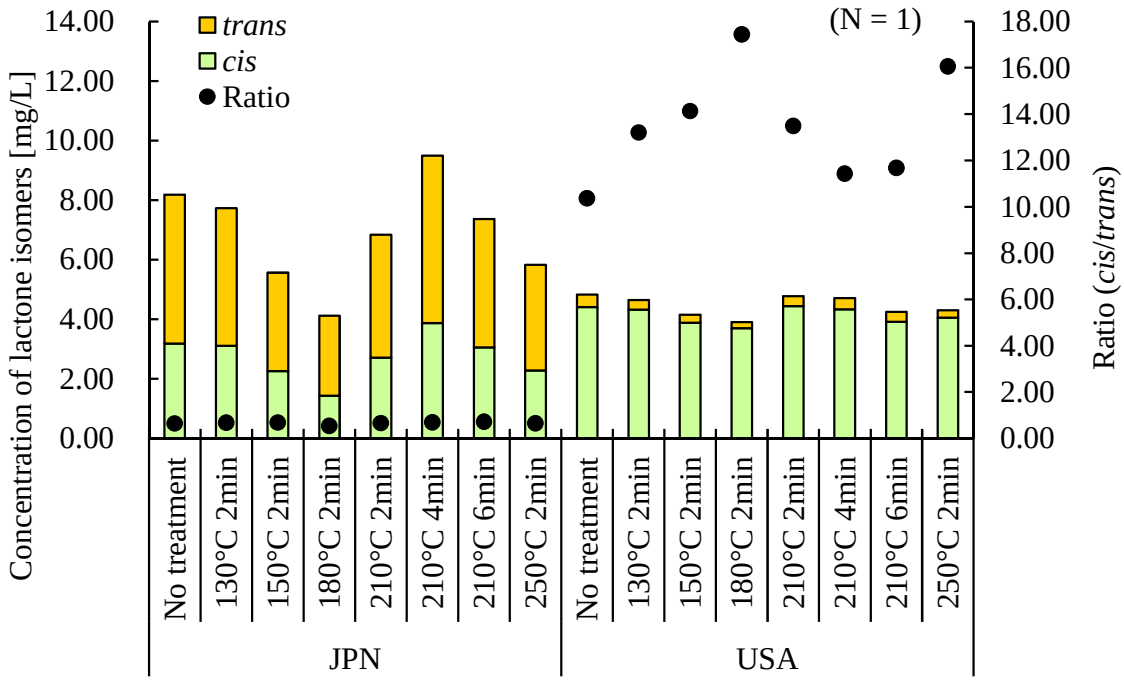


Figure 5.10 Effect of different heat treatments of stave slices on subsequent extraction of lactones into 60% abv solution

5.2.2.2 Stave regeneration in different depths of wood

In the previous section (**Section 5.2.2.1**), the effect of heat treatment of oak chips on lactone extraction was studied. However, within the distillery cooperage the actual heat treatment of the casks is done from the inner surface of staves and heat transmission is different from that of small wood chips due to their shapes and sizes (**Figure 5.11**).

Figure 5.11 Stave (left) and small wood chips (right)

In the following section, the heat treatment of staves was investigated further. The same staves as used previously in **Section 5.2.1.4**, were collected from a bottom stave of Japanese oak cask which had been used in whisky maturation for more than 30 years. The impact of heat treatments; light charring, heavy charring, or toasting using a handy burner was examined according to the method in **Section 2.2.3**. A series of 1 mm slice of wood chips were taken from different depths of the stave and were collected using a wood slicer according to the method in **Section 2.1.2** and the extraction using these chips was carried out by stirring with 60% abv spirit for one day at room temperature according to the methods in **Section 2.2.1**. The resultant samples of spirit were analysed for lactones (**Section 2.3.5**), colour (**Section 2.2.4**), and aromatics (**Section 2.3.4**). The data for lactones is presented in **Table 5.7** and the colour of the data in **Table 5.8** with the

aromatics in **Table 5.9**. The cumulative data are respectively presented in **Figures 5.12** to **5.18**.

When considering the effect of light charring, the concentration of total lactones at a depth of 1 mm was 1.75 mg/L and the highest found in the all conditions tested. However, highest concentration derived from light charring was observed at a depth of 20 mm (3.09 mg/L) and this was similar to the value obtained through heavy charring (3.02 mg/L) at the same depth of 20 mm. When the chips were toasted, the concentration at 1 mm was 0.76 mg/L and this was lowest observed in the all conditions, but highest concentration found was 3.63 mg/L at a depth of 19 mm and this was also highest observed for all conditions. When the ratios were compared, these were between 0.49 and 0.88 in for all depths and under all conditions, the only exception to this was for chips taken from a depth of 1 mm and subjected to light charring.

Although small differences were observed for all of the heat treatment conditions, similar trends in the concentration and ratio of lactones were identified. In addition to this, the depth of visual whisky soaking (9 mm) corresponds with an increase in total lactone at depth beyond this for all heat treatment conditions.

Depth (N = 1) [mm]	*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis*+*trans*) [mg/L as is]	Ratio (*cis*/*trans*)	*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis*+*trans*) [mg/L as is]	Ratio (*cis*/*trans*)
	No treatment				Light charring			
1	0.48	0.78	1.26	0.61	0.94	0.81	1.75	1.16
2	0.31	0.52	0.82	0.60	0.62	0.71	1.33	0.88
3	0.25	0.40	0.65	0.63	0.48	0.80	1.28	0.60
4	0.27	0.55	0.82	0.49	0.40	0.71	1.11	0.57
5	0.25	0.38	0.63	0.65	0.53	0.74	1.27	0.72
6	0.29	0.51	0.80	0.56	0.36	0.62	0.98	0.57
7	0.35	0.63	0.97	0.55	0.41	0.66	1.07	0.63
8	0.36	0.58	0.94	0.62	0.43	0.76	1.19	0.56
9	0.40	0.74	1.14	0.54	0.45	0.77	1.23	0.58
10	0.44	0.81	1.24	0.54	0.51	0.88	1.38	0.58
11	0.47	0.82	1.29	0.57	0.42	0.72	1.13	0.58
12	0.66	1.31	1.98	0.51	0.60	1.05	1.66	0.57
13	0.61	1.21	1.82	0.51	0.67	1.25	1.92	0.53
14	0.57	1.02	1.59	0.56	0.56	1.00	1.57	0.56
15	0.76	1.46	2.22	0.52	0.69	1.24	1.93	0.56
16	0.79	1.54	2.33	0.51	0.83	1.51	2.34	0.55
17	0.62	1.14	1.76	0.54	0.89	1.67	2.56	0.53
18	0.83	1.58	2.42	0.53	0.96	1.92	2.88	0.50
19	0.76	1.47	2.22	0.52	0.68	1.26	1.94	0.54
20	0.74	1.38	2.12	0.53	1.07	2.02	3.09	0.53
	Heavy charring				Toasting			
1	0.54	0.81	1.35	0.67	0.34	0.42	0.76	0.81
2	0.47	0.76	1.23	0.62	0.35	0.47	0.82	0.75
3	0.53	0.85	1.38	0.62	0.34	0.46	0.81	0.74
4	0.47	0.70	1.17	0.67	0.32	0.43	0.75	0.75
5	0.41	0.68	1.09	0.60	0.33	0.44	0.78	0.76
6	0.33	0.54	0.88	0.62	0.38	0.50	0.88	0.76
7	0.53	0.95	1.49	0.56	0.41	0.56	0.98	0.73
8	0.53	0.99	1.52	0.54	0.51	0.70	1.21	0.73
9	0.41	0.70	1.12	0.59	0.55	0.77	1.32	0.72
10	0.50	0.91	1.40	0.55	0.48	0.68	1.17	0.70
11	0.56	0.89	1.45	0.63	0.75	1.04	1.79	0.72
12	0.63	1.09	1.73	0.58	0.64	0.94	1.58	0.69
13	0.79	1.21	2.00	0.65	0.83	1.17	2.00	0.71
14	0.71	1.08	1.80	0.66	1.00	1.39	2.38	0.72
15	0.86	1.36	2.22	0.63	1.16	1.60	2.76	0.73
16	0.89	1.43	2.32	0.63	1.25	1.75	3.00	0.71
17	1.04	1.53	2.57	0.68	1.09	1.60	2.69	0.68
18	1.05	1.69	2.74	0.63	1.33	2.02	3.35	0.66
19	1.00	1.52	2.51	0.66	1.48	2.15	3.63	0.69
20	1.17	1.85	3.02	0.64	1.31	2.07	3.39	0.63

Table 5.7 Lactones in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

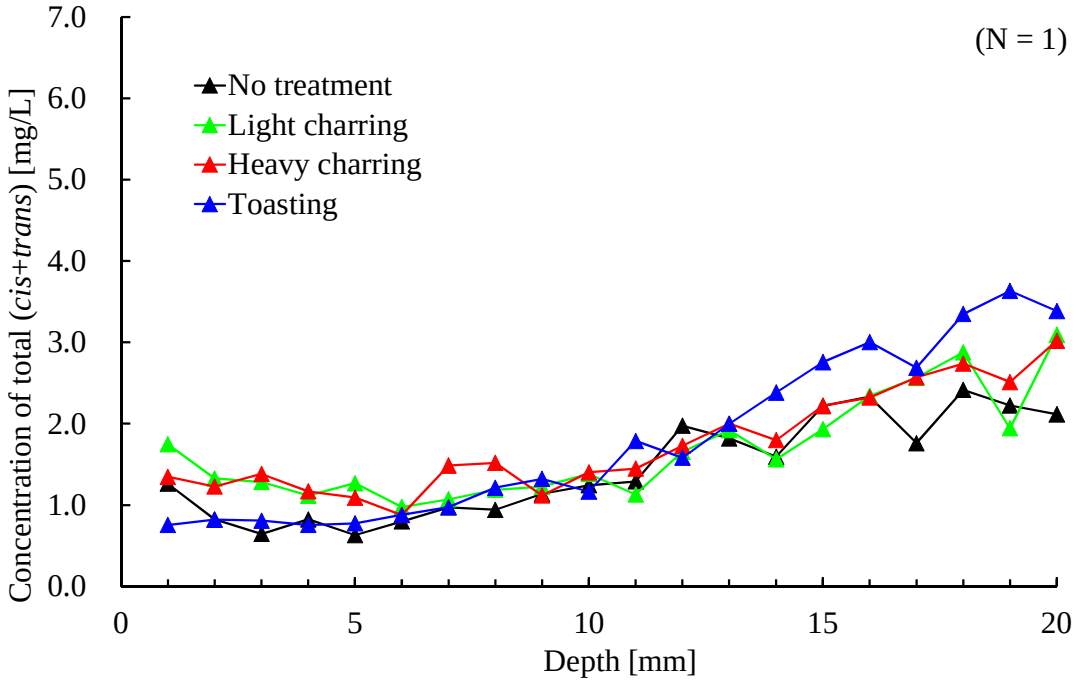


Figure 5.12 Lactones in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

From the result of the experimental work presented in **Table 5.8** and **Figure 5.13**, the colour was found to increase with a deeper stave depth, after 13 mm, the colour plateaued at 0.8 to 0.9 ABS, but the colours of depth of 1 to 5 mm were clearly lower. However, for all types of treatment the peak of colour extraction occurred between 7 mm and 9 mm. With no treatment the peak of colour extraction was between 8 mm and 12 mm. The toasting and heavy charring gave the highest, 1.12 and 1.13 ABS colour extraction, and light charring 1.10 ABS was in second.

	Colours [ABS]			
Depth (N = 1) [mm]	No treatment	Light charring	Heavy charring	Toasting
1	0.36	0.54	0.74	0.81
2	0.40	0.36	0.39	0.42
3	0.45	0.51	0.51	0.40
4	0.55	0.58	0.66	0.47
5	0.67	0.80	0.85	0.52
6	0.82	0.94	1.01	0.62
7	0.94	1.10	1.13	0.82
8	1.03	1.08	1.12	1.04
9	1.05	1.05	1.03	1.12
10	1.05	0.99	1.00	1.07
11	1.05	0.95	0.94	0.90
12	1.02	0.93	0.90	0.80
13	0.98	0.92	0.84	0.84
14	0.87	0.92		0.82
15	0.87	0.98	0.90	0.83
16	0.82	0.89	0.87	0.83
17	0.82	0.93	0.87	0.95
18	0.82	0.94	0.88	0.83
19	0.83	0.99	0.86	0.83
20	0.85	0.96	0.93	0.82

Table 5.8 Colour in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

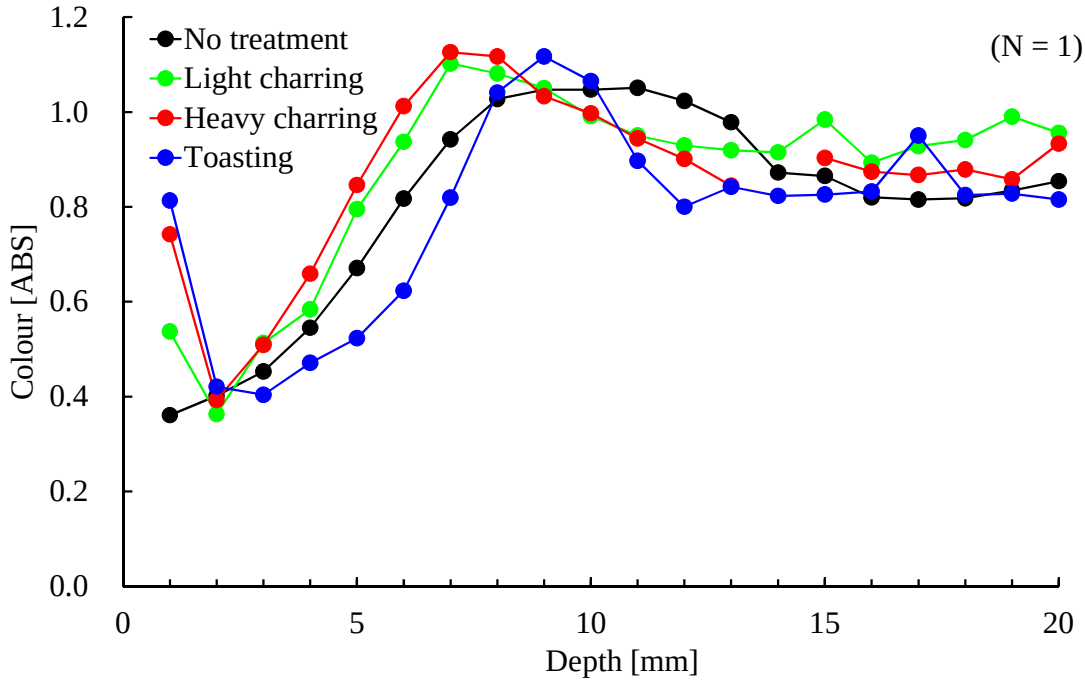


Figure 5.13 Colour in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

From the result presented in **Table 5.9** and **Figure 5.14**, it can be seen that vanillic acid at the shallower depths of 1 and 2 mm were found to have the highest values when considering all heat treatments, especially toasting (6.03 mg/L). Following these high levels of vanillic acid, the concentrations were subsequently found to decline with increasing stave depth. However, even with the decline in vanillic acid, these levels were still greater than those observed for the light and heavy charring treatments (**Figure 5.14**). Light and heavy charring treatments were found to increase the levels of vanillic acid in the shallow depth of 1 mm, (1.49 mg/L, 1.99 mg/L) compared to those observed at deeper depths of 2 to 4 mm (0.33 - 0.49 mg/L, 0.35 - 0.83 mg/L). From the heavy charring and light charring treatments, the concentration was suddenly increased to 1.58 and 1.42 mg/L from the depth of 5 mm. This may indicate generation by heat as these concentrations were greater than with no treatment.

Depth (N = 1) [mm]	Vanillic acid [mg/L as is]	Vanillin [mg/L as is]	Syringic acid [mg/L as is]	Syringaldehyde [mg/L as is]	Sinapaldehyde [mg/L as is]
	No treatment				
1	0.43	0.48	0.67	1.24	0.14
2	0.24	0.26	0.40	0.80	0.11
3	0.38	0.21	0.32	0.71	0.15
4	0.59	0.21	0.28	0.75	0.19
5	0.60	0.22	0.30	0.73	0.21
6	0.74	0.28	0.24	0.72	0.23
7	0.92	0.35	0.26	0.74	0.29
8	0.95	0.38	0.27	0.77	0.36
9	1.19	0.44	0.28	0.78	0.44
10	1.24	0.52	0.31	0.70	0.53
	Light charring				
1	1.49	3.42	2.95	10.03	11.58
2	0.49	0.69	0.70	1.23	1.12
3	0.43	0.65	0.51	1.01	0.35
4	0.33	0.53	0.35	0.75	0.32
5	1.42	0.61	0.38	0.76	0.34
6	1.69	0.63	0.53	0.79	0.42
7	1.94	0.65	0.67	0.80	0.51
8	1.95	0.63	0.74	0.80	0.58
9	2.10	0.65	0.95	0.86	0.66
10	1.95	0.64	1.03	0.86	0.71
	Heavy charring				
1	1.99	4.79	4.71	17.99	16.73
2	0.83	1.36	2.40	4.84	9.52
3	0.35	0.61	0.83	1.77	0.79
4	0.38	0.56	0.47	1.07	0.44
5	1.58	0.61	0.60	0.63	0.50
6	1.88	0.64	0.73	0.59	0.54
7	2.06	0.69	0.94	0.86	0.60
8	1.98	0.67	1.18	1.22	0.64
9	1.92	0.63	1.32	0.59	0.67
10	1.88	0.67	1.36	0.87	0.73
	Toasting				
1	6.03	19.11	11.22	46.83	103.78
2	3.64	7.65	8.57	22.94	87.82
3	2.33	3.76	6.32	11.80	57.42
4	1.71	2.53	4.71	8.23	40.08
5	1.34	2.15	3.86	8.10	28.84
6	1.05	1.94	3.41	8.56	16.99
7	0.86	1.72	3.10	8.13	8.15
8	0.70	1.54	2.80	7.20	4.35
9	0.69	1.27	2.25	5.51	2.89
10	0.60	1.15	1.75	3.83	2.24

Table 5.9 Aromatics in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

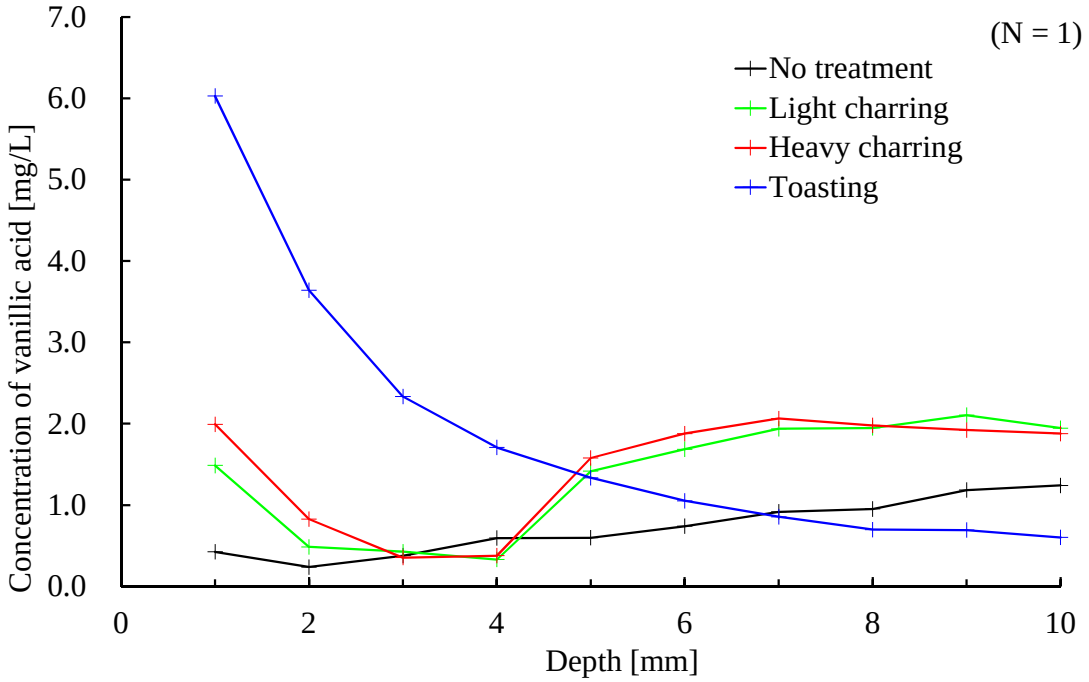


Figure 5.14 Vanillic acid in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

Examination of the concentrations of vanillin (**Table 5.9** and **Figure 5.15**) found that the shallower depths of 1, 2, and 3 mm showed higher levels with all heat treatments than those at deeper depths. The highest concentration observed was in the 1 mm toasted chips (19.11 mg/L). The concentration of vanillin observed following the toasting treatment remained higher than found with no treatment or, either of the charring treatments for all stave depths. However, the vanillin levels from the toasted samples declined from this initial peak (1 - 3 mm) and by 9 mm these values more closely matched those of the untreated samples and light and heavy charred chips.

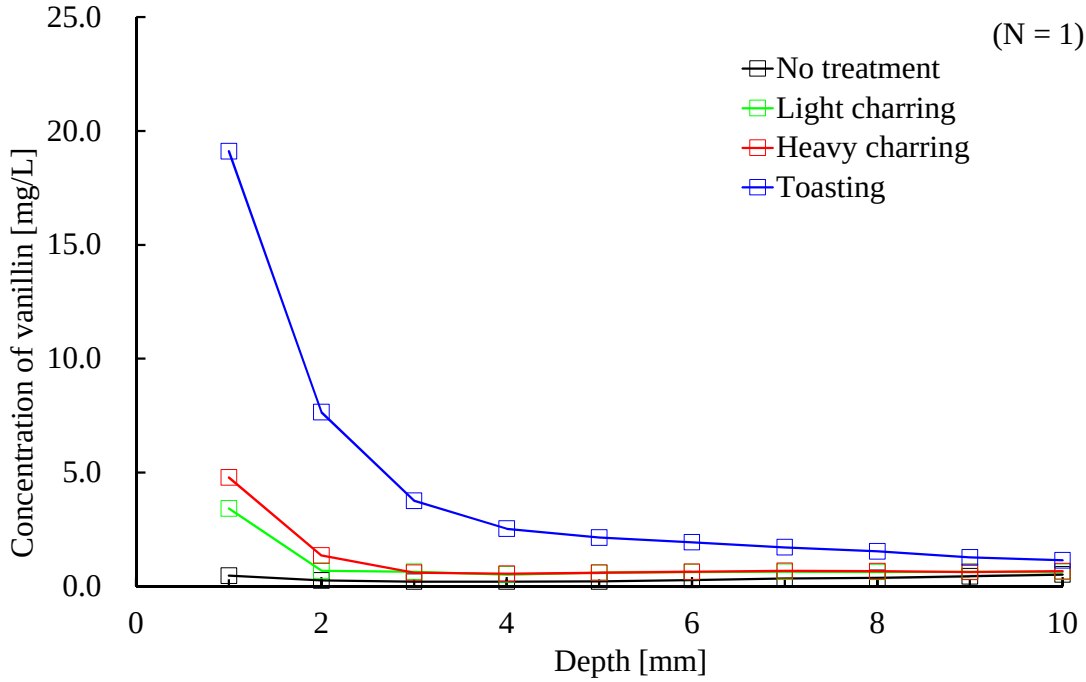


Figure 5.15 Vanillin in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

Evaluation of the results obtained for the syringic acid extraction (**Table 5.9** and **Figure 5.16**) found that shallower depths of 1 and 2 mm demonstrated higher levels for all heat treatments. The highest concentration observed was in the 1 mm toasted chips (11.22 mg/L). The concentration from treatment of wood chips by toasting remained higher for all stave depths. The concentration from light and heavy charring from the depth of 3 to 7 mm was low (0.35 - 0.94mg/L). This reflected the pattern previously observed with vanillin (**Figure 5.15**), although a slight difference was observed between all heat treatment conditions and no treatment in 10 mm.

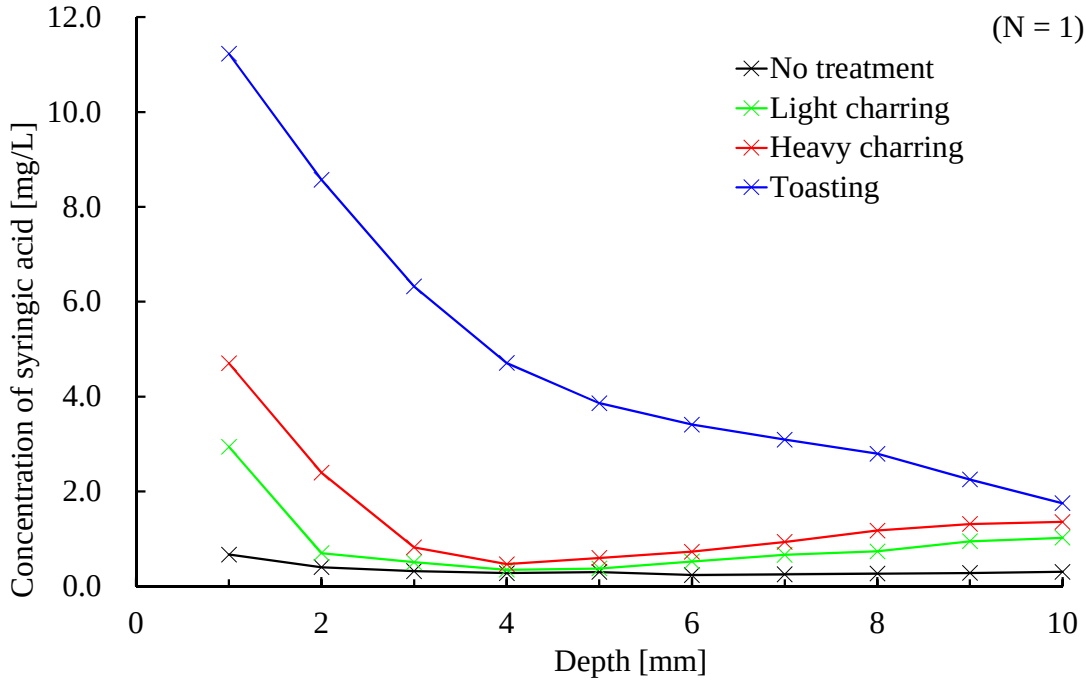


Figure 5.16 Syringic acid in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

The syringaldehyde results (**Table 5.9** and **Figure 5.17**) demonstrated that at shallower depths of 1, 2, and 3 mm extraction was greater for all the heat treatment conditions, especially toasting with a concentration of syringaldehyde with 1 mm chips of 46.83 mg/L. The concentration extracted from the toasted chips was greater at all depths of stave chips when compared to charred chips and the control untreated chips. This is a similar pattern to that observed for vanillin (**Figure 5.15**) and syringic acid (**Figure 5.16**).

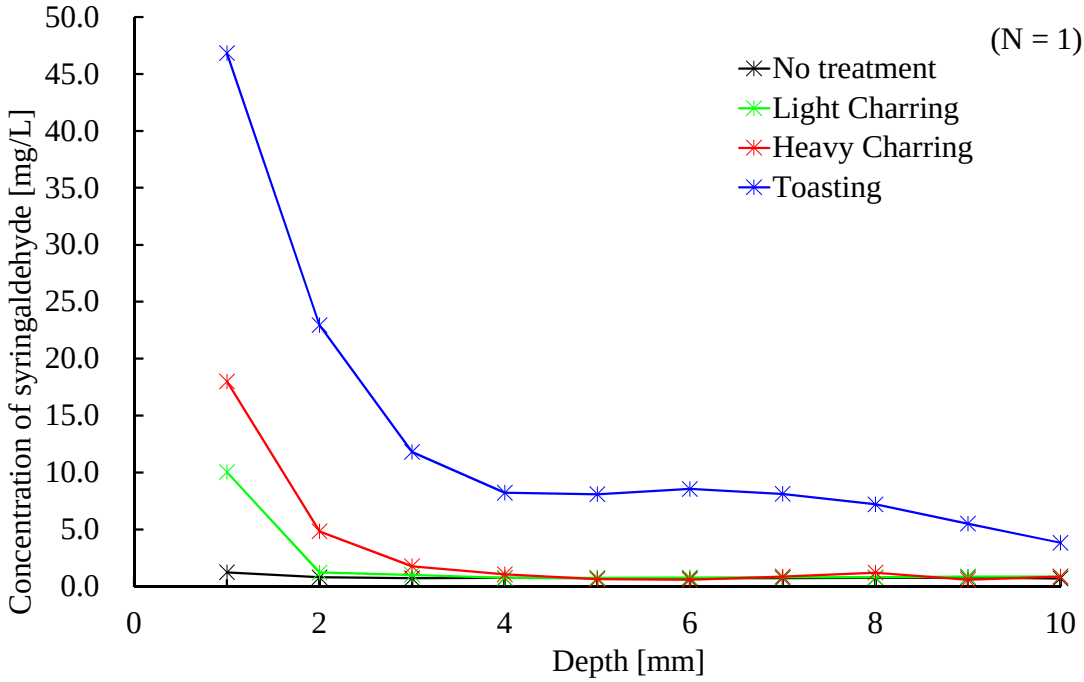


Figure 5.17 Syringaldehyde in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

The sinapaldehyde results (**Table 5.9** and **Figure 5.18**) demonstrated that extraction following toasting was much greater than for all other conditions and the concentration derived from the 1 mm chips was the highest (103.78 mg/L). The concentrations extracted toasted chips remained high in the deeper depths up to 10 mm. The concentration extracted from the toasted chips was greater at all depths of stave chips when compared to charred chips and the control untreated chips. This is a similar pattern to that observed for vanillin (**Figure 5.15**), syringic acid (**Figure 5.16**), and syringaldehyde (**Figure 5.17**).

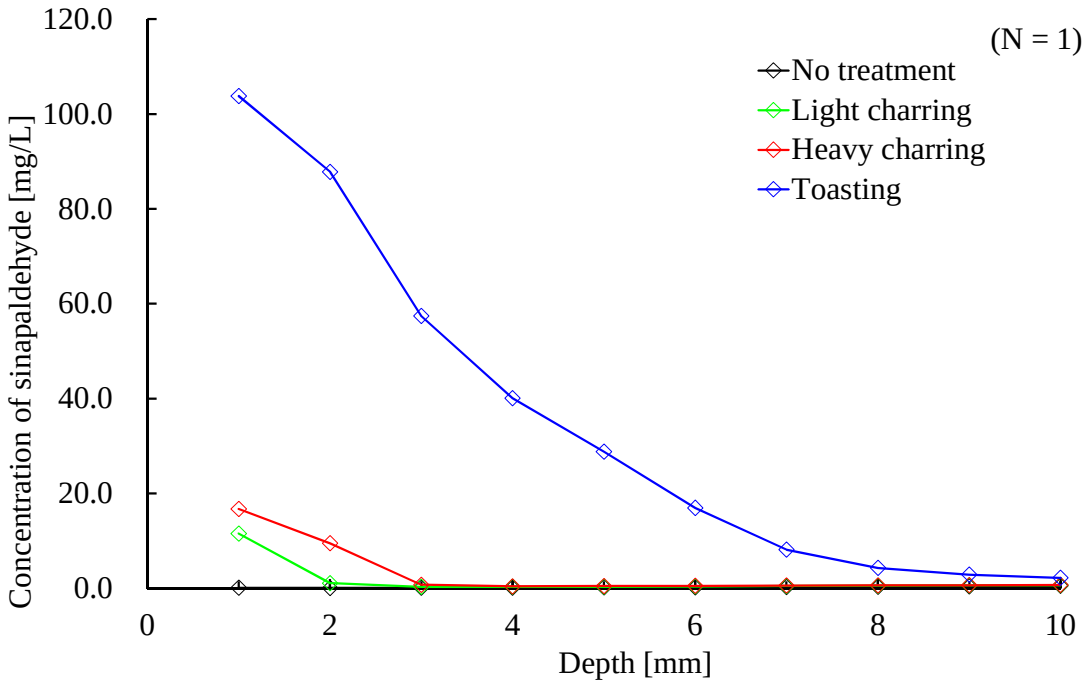


Figure 5.18 Sinapaldehyde in different depths of the Japanese oak stave after various heat treatments by extraction using 60% abv solution

The effects of toasting and charring of staves upon extraction of wood compounds into spirit was studied. The activation of extraction of lactones into spirit through heat treatment was not observed (**Table 5.7**). However, the regeneration of the colour and aromatics at the surface was demonstrated (**Table 5.8-9**, **Figure 5.13-18**). In particular, toasting gave deeper regeneration of aromatics, vanillic acid, syringic acid, and sinapaldehyde.

5.2.3 Whisky matured in re-charred cask

The effect of heat treatment on lactones was studied using Japanese oak chips (**Section 5.2.2.2**). In order to confirm the effect of heat treatment as a practical process, whiskies matured in production scale casks were analysed. Analysis for lactone and aromatics was carried out on young whiskies. These are Yamazaki malt whiskies had been

matured in Japanese oak cask for nine years in untreated refill casks or for ten years in re-charred refill casks (**Table 2.1** (**Section 2.1.1**)). Whiskies were sampled from seven different casks each for both cask types. Young whisky was used for this work due to cask availability with known provenance.

The results from the lactone analysis is presented in **Table 5.10**, with the cumulative data illustrated in **Figure 5.19**. When considering each lactone, *cis*-lactone concentrations were found to be very close in value (0.6 and 0.7 mg/L), and *trans*-lactones were also very similar (1.1 and 1.3 mg/L). The ratio of *cis*- to *trans*-lactones were the same at 0.6. Statistical analysis determined that there were no significant differences ($p = >0.05$) detected between the no treatment and re-charring (**Section 2.3**).

		cis lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
No treatment	Mean	0.6	1.1	1.7	0.6
	St. Dev.	0.1	0.4	0.4	0.2
Re-charring	Mean	0.7	1.3	2.0	0.6
	St. Dev.	0.1	0.3	0.3	0.1
p value by t-test (N = 7)		0.31	0.11	0.24	0.44

Table 5.10 Chemical analysis result of whisky lactones in young whiskies

(Raw data is available in Appendix 6)

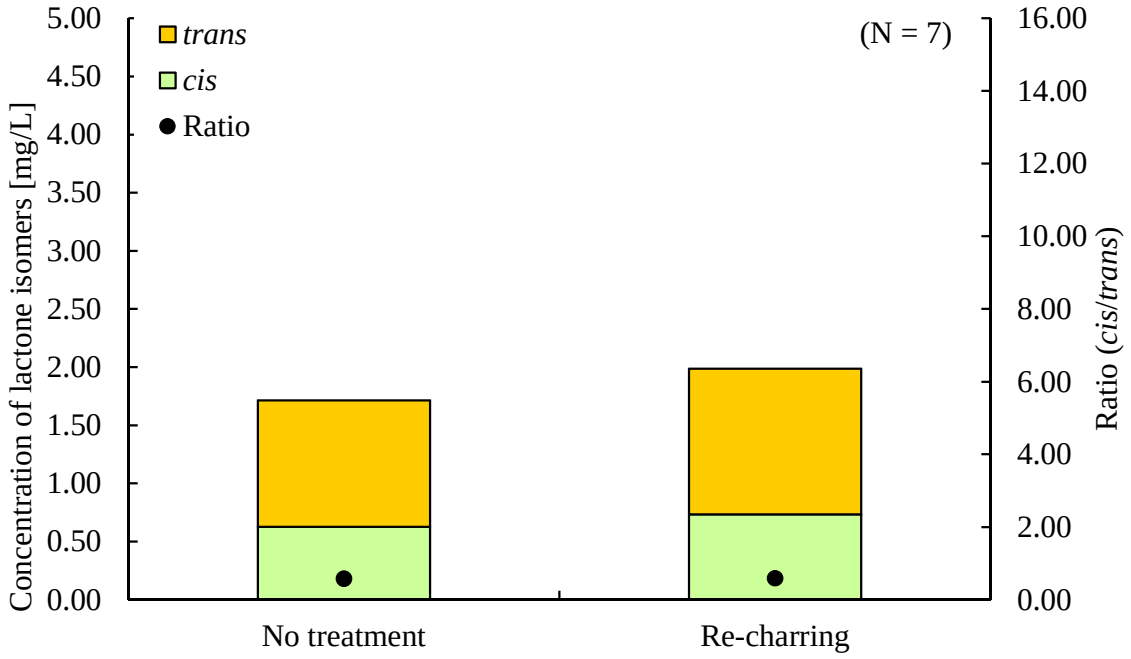


Figure 5.19 Chemical analysis result of whisky lactones in young whiskies

The data collected from the result of aromatics analysis shown in **Table 5.11** with the cumulative data illustrated in **Figure 5.20**. This data was found to reflect the patterns previously observed for vanillic acid and sinapaldehyde in the oak chips (**Section 5.2.2.2**). The concentration of aromatics extracted due to re-charring were slightly higher than with no treatment, 2.1 and 1.9 mg/L for vanillic acid, and 0.8 and 0.6 mg/L respectively for sinapaldehyde. When these data sets were compared to those for vanillin and syringic acid, the concentrations found in the spirit held in re-charred casks was found to be higher (3.6 and 2.5 mg/L) than where the cask had received no treatment (3.0 and 1.7 mg/L). In the case of syringaldehyde, the concentration in spirit from re-charred casks (6.3 mg/L) was much higher than spirit from casks with no treatment (4.3 mg/L). No significant differences were detected between the extraction levels of vanillic acid ($p = 0.18$). However the concentrations of syringic acid, vanillin, syringaldehyde, and sinapaldehyde were found to be significantly different ($p = 0.05$ or lower).

		Vanillic acid [mg/L as is]	Vanillin [mg/L as is]	Syringic acid [mg/L as is]	Syringaldehyde [mg/L as is]	Sinapaldehyde [mg/L as is]
No treatment	Mean	1.9	3.0	1.7	4.3	0.6
	St. Dev.	0.3	0.5	0.2	0.7	0.1
Re-charring	Mean	2.1	3.6	2.5	6.3	0.8
	St. Dev.	0.3	0.5	0.3	0.6	0.1
p value by t-test (N = 7)		0.18	0.05	<0.01	<0.01	<0.01

Table 5.11 Chemical analysis result of aromatics in young whiskies

(Raw data is available in Appendix 7)

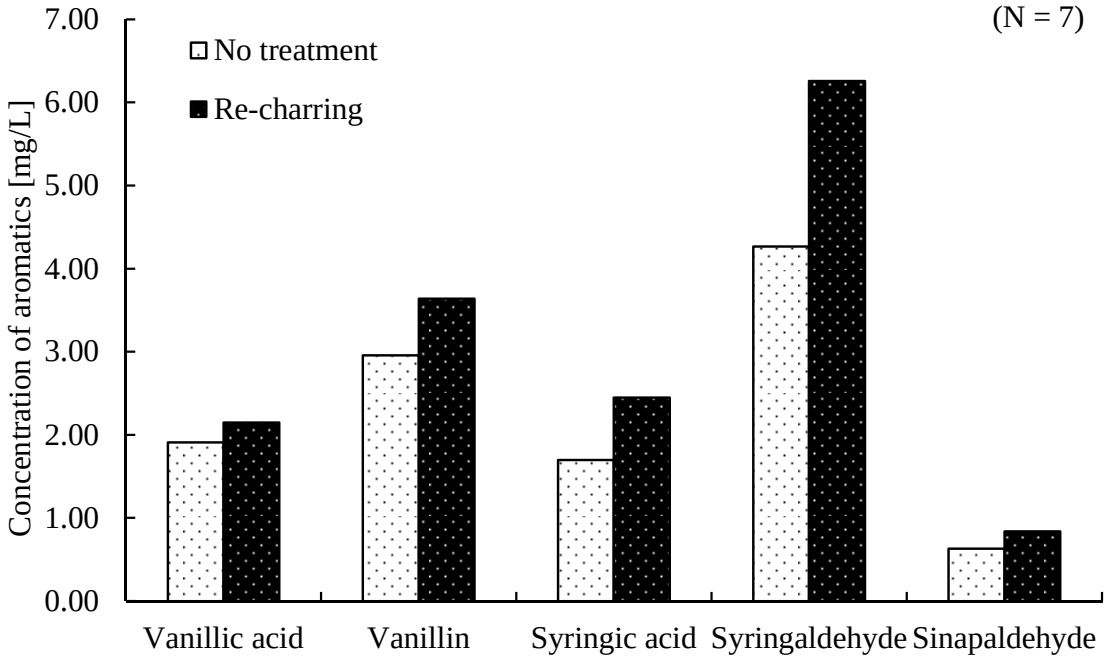


Figure 5.20 Chemical analysis result of aromatics in young whiskies

5.3 Discussion

The factors which influence the release of lactones from the oak staves of casks during maturation were studied using the wood chip extraction using the ethanol solution methodology developed earlier in this study.

When the stave position was compared, all stave extractions demonstrated the same amount and ratio of lactones (**Section 5.2.1.3**). This was surprising as it had been postulated that contact with maturing spirit over long periods of time would have had an impact on the compounds remaining in the wood chips (**Section 1.1.2.1**). It was clear that there was no difference in the stave position within the cask from this study. It is suggested that one reason for this could be that wood chips were collected from the outside surface of cask and penetration or extraction did not reach to the outside surface and the outside surface was not influenced by the maturation inside the cask. Comparison of the ratios of sample 'top', 'middle', and 'bottom' were similar 0.68, 0.72, 0.77, however the value for the 'all' sample was 0.49 not as expected, which would have been to give a value around the middle of values the other positions (**Table 5.3**). It is suggested that a reason for this may be that the 'all' sample were not a true mixture of these three positions, but collected from a selection of the all staves of the cask used in this work.

The impact of the contact of spirit penetrating to differing depths of staves in casks was investigated (**Section 5.2.1.4**). The data collected demonstrated that the lactones and most of the aromatics were extracted from the wood chips consistently with no apparent relationship with depth of the whisky soaking (visual observation) achieved by 30 years of storage and maturation. No differences were determined in the lactones ratio (**Table**

5.4). If the staves of old casks are sliced and analysed, it can be confirmed what amount of compounds is still remained in the cask without waiting for further maturation. Therefore, the analysis of sliced staves could be used to predict how long or how many times the cask can be used as an active cask from wood extracts point of view, and be part of a wood policy.

The effects of various heat treatments on wood chips and their subsequent extraction were studied (**Section 5.2.2.1** and **Section 5.2.2.2**) using Japanese oak and American oak chips. The total amount of lactone which was extracted from Japanese oak were not found to be consistent, but the concentration of lactone extracted from American oak were consistent across the conditions tested (**Table 5.6**). It is not clear why the results of Japanese oak were not consistent and this requires further investigation (**Section 5.2.2.1**). The more intense heat treatments of toasting and heavy charring showed higher colour, confirming that the stave surface was influenced by heat. In the toasting, the depth of highest colour was 2 mm deeper those of than the light and heavy charring. The reasons behind this are unclear, but one possibility is that the residue which was observed in 9 - 11mm may be pushed deeper into the wood during longer periods of heat treatment (**Section 5.2.2.2**).

The heat regeneration in different depths of stave by light charring, heavy charring, and toasting were studied. Considering the colour, the more intense heat treatments of toasting and heavy charring showed higher colour (**Table 5.8**). This result indicated that the surface of the stave was influenced by heat. Also, the lower colour values one to five mm suggest that this is the depth to which colour compounds are leeched from the stave during a 30 year period of maturation. Considering aromatics, these concentrations following toasting remained higher for all stave depths (**Table 5.9**). However, at a greater depth these concentrations more closely matched those of the

untreated, lightly and heavily toasted chips. These are probably caused by the strength of heat treatment and heat transmission respectively. Although regeneration of the colour and aromatics near the surface was observed, heat treatment of the wood chips and staves showed no activation of lactones, with both the levels and their ratio being unaffected (**Table 5.7**). All aromatics at the shallower depth of 1 and 2 mm was found to have the highest values when considering all heat treatments, especially toasting (**Table 5.9**). It is suggested here that toasting is the optimum heat treatment for the generation of aromatics.

Finally, the whiskies, of the almost same age, matured in either un-treated or re-charred casks were compared (**Section 5.2.2.3**). The whiskies matured in re-charred casks showed elevated concentrations of aromatics (**Table 5.11**). Again no significant difference in lactone levels was observed (**Table 5.10**). This result using matured whisky supported the result using model solutions using oak chip (**Section 5.2.2.2**).

Heat treatment is normally used for the regeneration of cask in order to add a colour and aroma (**Section 1.3**). Colour level and aromatic compound concentration were observed to increase with heat treatment. However, lactones which are the important aroma compound for Japanese oak character in whisky were not activated by any of the heat treatments under investigation. Therefore it is suggested that the regeneration of casks by heat treatment is limited, and is not effective for the activation of the Japanese oak character which is a desirable and unique character of Japanese oak whisky.

From these results, two important learnings can be made. One is that surface shaving of the insides of the cask would not to be sufficient for the activation and extractions of lactones and aromatics by future spirit fills. The second one is that the aromas derived

from aromatics (vanilla, woody, etc.), are enhanced by heat treatment, while the coconut aroma from lactone is not. In other words, the heat treatment during cask regeneration is selective. Some aroma compounds such as vanillin are generated through heat treatment while others, such as the lactones, are naturally present in the oak. Once the levels of the latter are depleted by a period of maturation they cannot be regenerated.

Chapter 6: Synergy between whisky lactone isomers on coconut aroma

6.1 Introduction

In work presented earlier (**Section 4.2.1**), it was determined by sensory analysis that one of the typical aromas of JPN whisky could be described as 'coconut'. In addition, in this study chemical analysis focussed on the coconut aroma compound 'whisky lactone', and it was found that Japanese oak had a higher concentration of *trans*-lactone than other oaks (American or European). However, it was also found that the total lactone content of Japanese oak whisky was similar to the levels expressed in USA whisky (**Section 4.2.2**). It has been postulated, as a result of this work, that the *cis*/*trans* lactone ratio may provide a means of identifying, through analytical methods, whether or not a whisky has been matured in Japanese oak.

The work presented in this thesis has developed that lactones are an important coconut aroma component of JPN whisky. Following on from this two questions were posed, why do JPN whiskies have a coconut aroma? And, does *trans*-lactone have any significant aroma effect?

In this chapter, sensory analyses of threshold measurement and lactone isomer addition to model solutions or whiskies were carried out in order to study any effects of *trans*-lactone on coconut aroma in whisky. In addition to this, a numerical relationship was investigated to explain the effect.

6.2 Results

6.2.1 Determination of odour thresholds of lactones

The two naturally occurring isomers (3S,4S (*cis*), 3S,4R (*trans*)) of whisky lactone have slightly different aromas (Koppenhoefer et al., 1994), but they all have coconut notes and could potentially contribute to coconut aroma in whisky (**Section 4.2.2**). In order to determine the odour thresholds for each lactone, the threshold of *cis*-lactone, *trans*-lactone, and *cis* and *trans*-lactone 1:1 mixture in 20% abv ethanol were calculated using the method in **Section 2.4** (ISO 13301) (**Table 6.1**). The total number of panellists were plotted against log concentration. A straight line was fitted to this graph, and the formula of this line, $y = \mathrm{A}x + \mathrm{B}$, was calculated by the least squares method. This was performed for *cis*-lactone (**Figure 6.1**), *trans*-lactone (**Figure 6.2**) and the 1:1 ratio mixture (**Figure 6.3**). The Log concentration at which half the panel can detect the aroma was calculated and this was converted back to the standard concentration (**Table 6.1**).

	Concentration [mg/L]	Log concentration	Number detecting aroma	Total number of panelists	Straight line formula values		Threshold [mg/L]
					A	B	
cis lactone	0.05	-1.3010300	2	2	26.51	36.86	0.15
	0.10	-1.0000000	8	10			
	0.15	-0.8239087	7	17			
	0.20	-0.6989700	1	18			
	0.25	-0.6020600	2	20			
	N.D.		10	30			
trans lactone	0.40	-0.3979400	6	6	33.32	17.26	0.83
	0.80	-0.0969100	4	10			
	1.20	0.0791812	10	20			
	1.60	0.2041200	6	26			
	2.00	0.3010300	0	26			
	N.D.		3	29			
cis and *trans* lactone 1:1 mixture	0.05	-1.3010300	2	2	39.23	52.12	0.11
	0.10	-1.0000000	8	10			
	0.15	-0.8239087	12	22			
	0.20	-0.6989700	3	25			
	0.25	-0.6020600	3	28			
	N.D.		2	30			

Table 6.1 Determination of odour thresholds of lactones in 20% abv ethanol solution

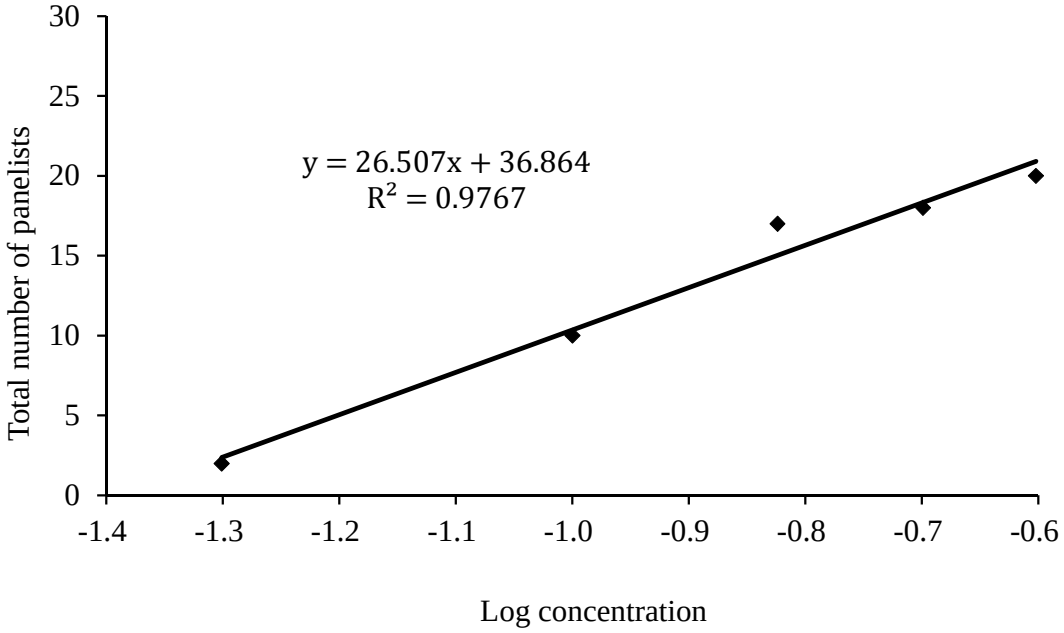


Figure 6.1 Threshold calculation of *cis*-lactone in 20% abv ethanol solution

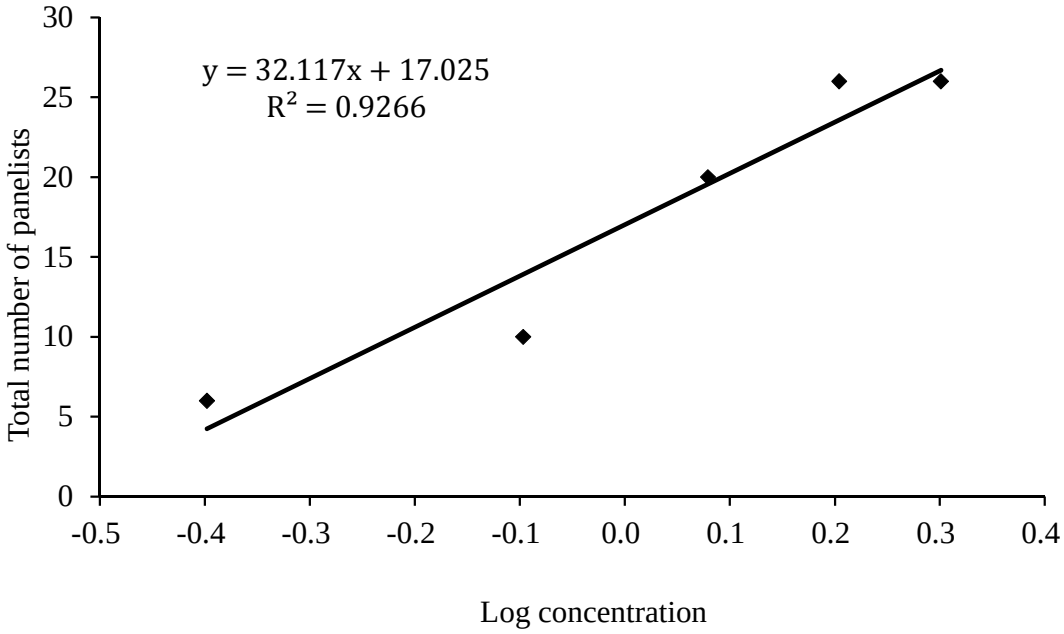


Figure 6.2 Threshold calculation of *trans*-lactone in 20% abv ethanol solution

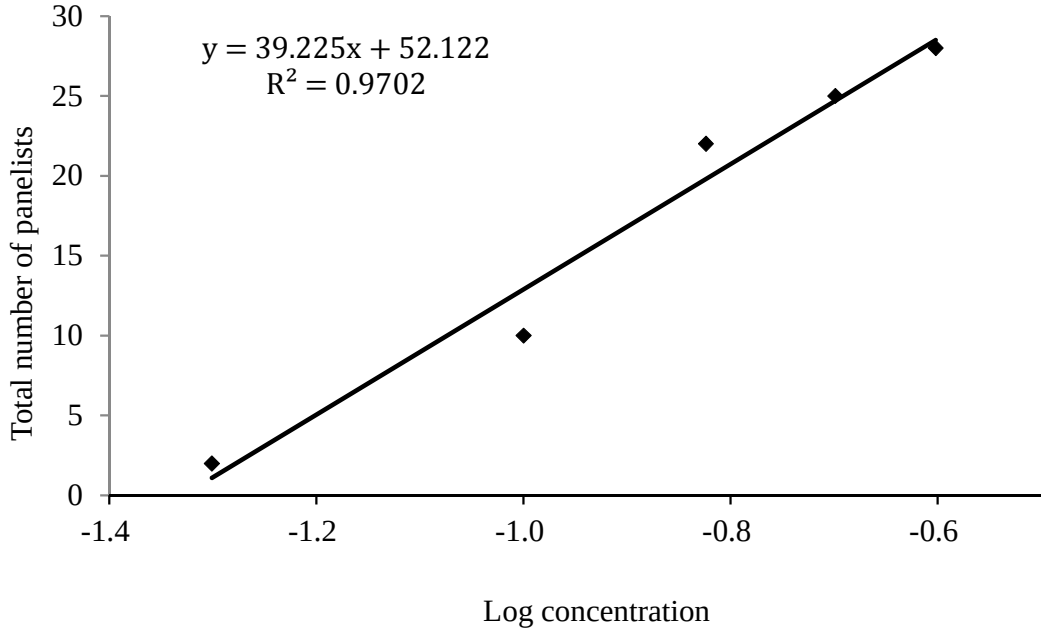


Figure 6.3 Threshold calculation of *cis* and *trans*-lactone 1:1 mixture in 20% abv ethanol solution

The threshold of *cis*-lactone was 0.15 mg/L, that of *trans*-lactone was 0.83 mg/L, and the threshold of the 1:1 mixture was 0.11 mg/L. The R^2 (correlation coefficient) of *cis*-lactone and 1:1 mixture were slightly higher than that of the *trans*-lactone, however all values showed good correlations (greater than 0.9). These results demonstrate that the threshold of *cis*-lactone was 5.5 times lower than that of *trans*-lactone, and is close a previous study (Otuska et al., 1974) carried out in 30% abv ethanol solutions. This previous study determined the thresholds to be *cis*-lactone was 0.067 mg/L and that of *trans*-lactone was 0.79 mg/L. In similar research using wine, the threshold of each lactone was reported by Brown et al. (2006). These authors reported that *cis*-lactone had a lower threshold (0.057 mg/L) than *trans*-lactone (0.38 mg/L). In the past, due to the higher ratio of *cis*-lactone in American oak European oaks, and the lower threshold of *cis*-lactone, most studies have focussed on this isomer (Waterhouse et al., 1994; Diaz-Plaza et al., 2002; Fernandez et al., 2003; Brown et al., 2006).

It was additionally observed in this study (**Table 6.1**) that the threshold of the 1:1 mixture containing both lactones (0.11 mg/L) was similar to that of either *cis*-lactone (0.15 mg/L) and lower than *trans*-lactone (0.83 mg/L) when measured individually. Therefore it was postulated that there is a synergistic effect between the *cis*- and *trans*-lactones.

6.2.2 Synergistic effects on aroma between whisky lactones

6.2.2.1 Addition of lactones to whisky

The influence of each lactone in the complex matrix of whisky (compared to 20% abv ethanol) was a subsequent focus of this research with further studies adding each lactone to whisky. The spirits used for this work were 20 year old JPN and USA whiskies as these contained different original ratios of lactones (**Section 4.2.2**). To these whiskies 1 mg/L of each lactone was added (**Section 2.1.6**), and the sensory analyses (**Section 2.4.1-3**) were carried out with specific focus on the coconut descriptor. The sensory results obtained when each of the lactones were added to the USA and JPN whiskies are given in **Table 6.2** illustrated in **Figure 6.4**.

When *trans*-lactone was added to the USA whisky, intensity increased more (from 0.4 to 0.9) than *cis*-lactone (from 0.4 to 0.7). This pattern was not observed in the JPN whisky sample. When either *cis*- or *trans*-lactones were added a similar increase in intensity (from 0.5 to 1.0 and 1.1 respectively) was observed. This increase was greater than detected in the USA whisky. These differences in JPN whiskies were determined to be significantly different ($p = 0.03, 0.02$) (**Table 6.2**).

		No addition	*cis* addition	*trans* addition
USA-20yo	Mean	0.4	0.7	0.9
	St. Dev.	0.3	0.6	0.6
	p value by t-test (N = 14)	-	0.14	0.02
JPN-20yo	Mean	0.5	1.0	1.1
	St. Dev.	0.3	0.7	0.8
	p value by t-test (N = 13)	-	0.03	0.02

Table 6.2 Sensory results of addition experiment to USA and JPN 20yo whiskies using coconut descriptor

(Raw data is available in Appendix 8, 9)

When *trans*-lactone or *cis*-lactone was added, all of intensities of coconut aroma in JPN whisky increased to similar levels (**Figure 6.4**).

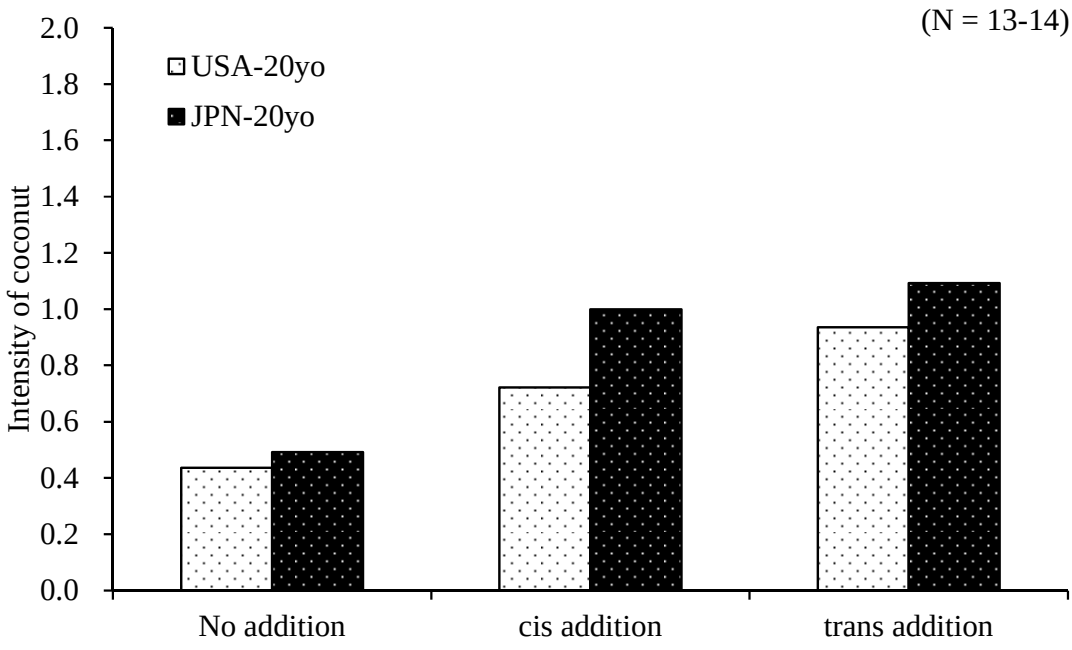


Figure 6.4 Sensory results of addition experiment to USA and JPN 20yo whiskies using the 'coconut' descriptor

6.2.2.2 Study of coconut aromas in model ethanol solutions

Model solutions were prepared using 20% abv ethanol spiked with additional lactones which contained either no lactone or 1.00 mg/L of each lactone in order to examine the aroma activities of each lactone (*cis*- or *trans*-) in a simple model solution (**Section 2.1.5**). Sensory analysis was carried out according to the method described in **Section 2.4.1-3**. The results of this analysis are summarised in **Table 6.3**. The intensity of *cis* addition was given a value of 1.3 and *trans* addition a value of 0.5. The model solutions demonstrated that addition of *trans*-lactones did not produce a significant increase in the detection of coconut aroma. Addition of the *cis*-lactone resulted in the detection of coconut aroma which was significantly different ($p = <0.01$) to the ethanol with no additions.

		Amount [mg/L as is]			Intensity
		cis lactone	*trans* lactone		
1.00 mg/L model solution	No addition	0.00	0.00	Mean	0.3
				St. Dev.	0.4
	cis addition	1.00	0.00	Mean	1.3
				St. Dev.	0.8
				p value by t-test (N = 15)	<0.01
	trans addition	0.00	1.00	Mean	0.5
				St. Dev.	0.6
				p value by t-test (N = 15)	0.29

Table 6.3 Intensity of coconut aroma in samples of 20% abv ethanol model solutions containing each lactone

(Raw data is available in Appendix 10)

Following on from this, ethanol solutions (20% abv) spiked with additional lactones were prepared to replicate the lactone levels as were found in the 20 year old JPN and USA whiskies (**Section 2.1.5**; **Table 4.4**). These solutions also underwent sensory analysis (**Section 2.4.1-3**) for the intensity of the coconut aroma of interest to this research (**Table 6.4**). **Figure 6.5**, which is cumulative data of **Table 6.3** and **Table 6.4**, illustrates that *cis*- addition resulted in a higher sensory mean score of (1.6) in the coconut aroma, this difference was found to be significant ($p = 0.04$). This pattern is similar to that observed with 1.00 mg/L model solution. When considering the *trans*- addition, this resulted in greater detection (with a sensory mean score of 1.5) which was same level of *cis*- addition (mean score of 1.6). This did not reflect the observations made using the 1.00 mg/L model solution. These results which found that both *cis*- and *trans*- additions resulted

in greater sensory intensity was quite similar with that demonstrated by JPN whisky in **Figure 6.4**.

		Amount [mg/L as is]			Intensity
		cis lactone	*trans* lactone		
JPN-20yo model solution	No addition	0.41	0.82	Mean	0.9
				St. Dev.	0.3
	cis addition	1.41	0.82	Mean	1.6
				St. Dev.	0.8
				p value by t-test (N = 8)	0.04
	trans addition	0.41	1.82	Mean	1.5
				St. Dev.	0.8
				p value by t-test (N = 9)	0.07
USA-20yo model solution	No addition	1.01	0.10	Mean	1.0
				St. Dev.	0.7
	cis addition	2.01	0.10	Mean	1.5
				St. Dev.	0.6
				p value by t-test (N = 8)	0.15
	trans addition	1.01	1.10	Mean	1.5
				St. Dev.	0.7
				p value by t-test (N = 9)	0.14

Table 6.4 Intensity of coconut aroma in samples of 20% abv ethanol model solutions on replicating the lactone isomers of JPN or USA 20yo whisky (Raw data is available in Appendix 11)

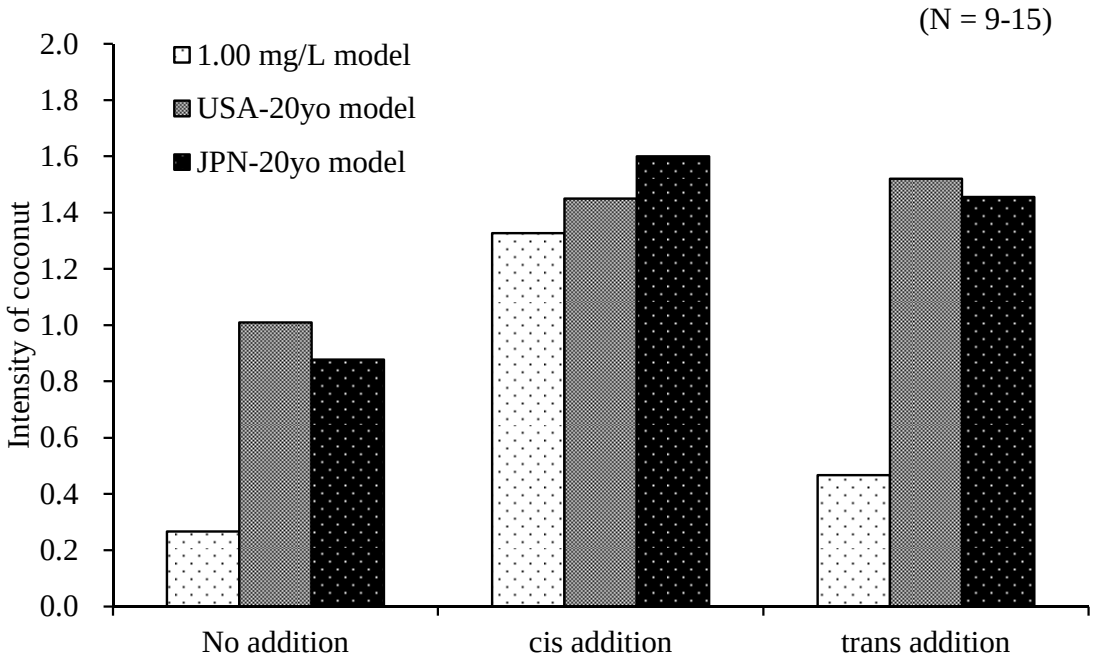


Figure 6.5 Intensity of coconut aroma in samples of 20% abv ethanol model solutions on replicating of USA and JPN 20yo whiskies

6.2.2.3 Coconut aroma intensity

Following on from the previous work presented in this chapter (**Section 6.2.2.1**) it may be said to be surprising that 'coconut' is a typical aroma of JPN whisky (**Section 4.2.1**) when these whiskies show higher amounts of *trans*-lactone, which is less aroma active, than *cis*-lactone. This observation merited further investigation, therefore in order to examine this coconut aroma, the calculated coconut dose over threshold of predicted coconut aroma intensity was defined using threshold of each lactone. Equation 1 [**EQ1**] was constructed by using the sum of coconut aroma intensity for each lactone isomer. It is suggested that this could be used to predict coconut aroma based on a combination of the concentrations (**Figure 6.5**) and thresholds of the two lactones (**Section 6.2.1**).

$$\text{Calculated coconut dose over threshold} = \frac{[cis]}{0.15} + \frac{[trans]}{0.83}$$

[EQ1]

Using formula **EQ1**, the dose over threshold of *cis*-lactone (1.00 mg/L) is calculated to be 6.7, and of the *trans*-lactone (at a concentration of 1.00 mg/L) is 1.2. Unsurprisingly the dose of the *trans*-lactone 1.00 mg/L solution is lower than that of the *cis*-lactone 1.00 mg/L solution. Results of the sensory evaluation, shown in **Table 6.4**, agree with the calculated doses (**Table 6.5**) although it should be noted that these calculated doses and the nosing scores had different scales.

Sample	Amount [mg/L as is]		Calculated dose over threshold	Nosing score
	cis lactone	*trans* lactone		
cis -lactone	1.00	0.00	6.7	1.3
trans -lactone	0.00	1.00	1.2	0.5

Table 6.5 Study of coconut intensity using calculated dose over threshold for 20% abv ethanol solutions including each lactone isomer

Following on from the application of **EQ1** to the model solutions it was applied to 20 year old USA and JPN whiskies (**Section 2.1.1**), based on the levels of each of the lactones that they contained (**Table 4.4**). The calculated dose of JPN whisky was 3.7, which was about half that of USA whisky with a score of 6.8. However, the sensory results (**Figure 4.1**) showed the inverse of this, with the JPN whisky being given higher scores for this aroma (**Table 6.6**).

Sample	Amount [mg/L as is]		Calculated dose over threshold	Nosing score
	cis lactone	*trans* lactone		
USA-20yo	1.01	0.10	6.8	0.8
JPN-20yo	0.41	0.82	3.7	1.4

Table 6.6 Coconut intensity and sensory result in whisky using calculated dose over threshold

In order to examine this unexpected difference between calculated dose and actual coconut intensity, the influence of each lactone in the whisky matrix became the focus of the investigation. The experiments were carried out using the 20 year old USA and JPN whiskies (**Section 2.2.2**), which contained different original ratios of lactones. In the first experiment 1.00 mg/L of each lactone was added to the USA whisky. The calculated dose was lowest in the sample containing no added lactone (score of 6.8). The highest dose (13.5) was found in the sample with added *cis*-lactone, and an intermediate dose (8.1) was found in the one with added *trans*-lactone (**Table 6.7**). However, these calculated doses did not reflect the nosing scores gathered from the sensory panel. This held true for both the USA and JPN whiskies. The nosing scores of both the USA and JPN whiskies with added *trans*-lactone were highest. This result indicated that the *trans*-lactone has higher coconut aroma activity than *cis*-lactone.

Sample	USA-20yo				JPN-20yo			
	Amount [mg/L as is]		Calculated dose over threshold	Nosing score	Amount [mg/L as is]		Calculated dose over threshold	Nosing score
	cis lactone	*trans* lactone			*cis* lactone	*trans* lactone		
No addition	1.01	0.10	6.8	0.4	0.41	0.82	3.7	0.5
cis 1 mg/L	2.01	0.10	13.5	0.7	1.41	0.82	10.4	1.0
trans 1 mg/L	1.01	1.10	8.1	0.9	0.41	1.82	4.9	1.1

Table 6.7 Coconut intensity and sensory results for additional experiment using calculated dose over threshold

6.2.2.4 Possibility of a synergistic effect between whisky lactones

In order to examine why the *trans*-lactone shows this high aroma activity, 20% abv ethanol model solutions were prepared which contained the same concentrations of each lactone as the 20 year old JPN whisky used in **Section 6.2.2.2**. The sensory results for these model samples (**Table 6.4**) are shown in **Figure 6.6**, alongside the original whisky sensory results (**Table 6.2**). In the model samples, *cis*- and *trans*- addition showed similar scores of 1.5, which was higher than the score of 0.9 for no addition. In the JPN whisky, *cis*- and *trans*- addition showed also the mostly same score of 1.0 which was higher than the score of 0.5 for no addition.

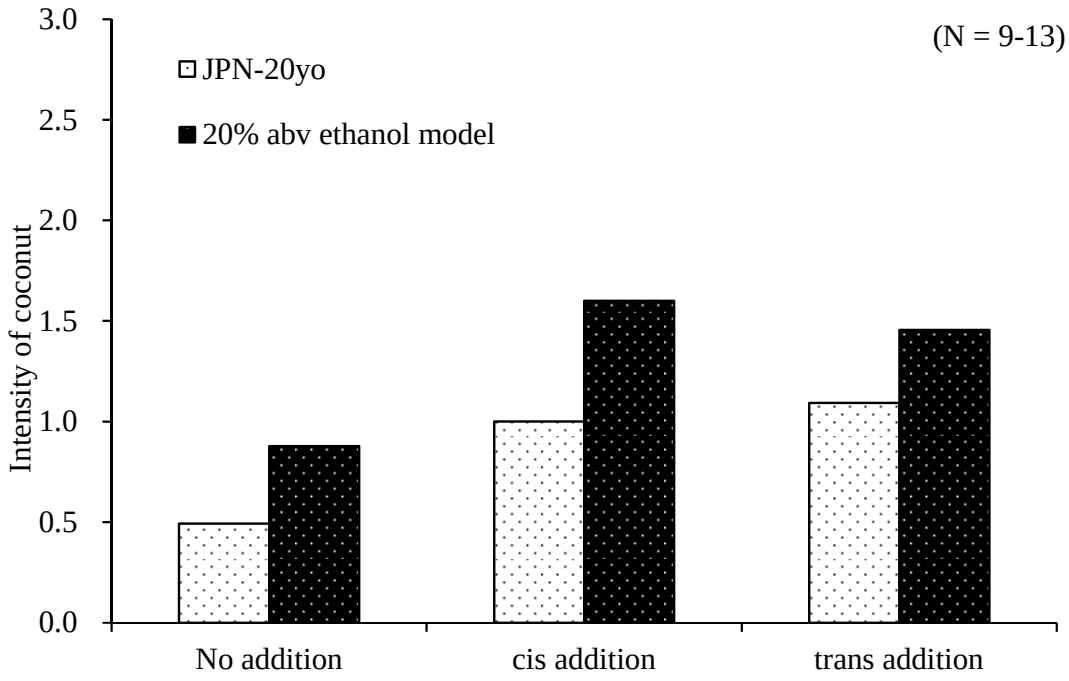


Figure 6.6 Comparison of sensory results of JPN whisky and 20% abv ethanol model samples replicating of JPN whisky

6.2.2.5 Numerical analysis for synergistic effects

The results of the previous section (**Section 6.2.2.4**) suggest a relationship between the ratio of lactones and the aroma activity detected during sensory analysis. The possibility of a numerical relationship to explain or more accurately predict the aroma intensity was explored by further sensory analysis using model solutions containing lactone isomers.

6.2.2.5.1 Sensory analysis of individual lactone isomers

The first requirement was to determine the intensity of 'coconut' aroma in the 20% abv ethanol model solutions containing varying amounts of the each lactones. The concentration of lactone studied were 0.25, 0.50, 0.75 and 1.00 mg/L. The samples

underwent sensory analysis (**Section 2.4.1-3**) the results are shown in **Table 6.8** and illustrated in **Figure 6.7**.

		Concentration of lactone [mg/L]			
		0.25	0.50	0.75	1.00
cis lactone	Mean	0.7	1.2	1.7	2.0
	St. Dev.	0.4	0.4	0.5	0.5
	p value by t-test (N = 12)	-	<0.01	<0.01	<0.01
trans lactone	Mean	0.2	0.3	0.6	0.8
	St. Dev.	0.3	0.4	0.6	0.7
	p value by t-test (N = 12)	-	0.58	0.13	0.02

Table 6.8 Intensity of coconut aroma in 20% abv ethanol solutions containing various amounts of either *cis* or *trans*-lactone

(Raw data is available in Appendix 12)

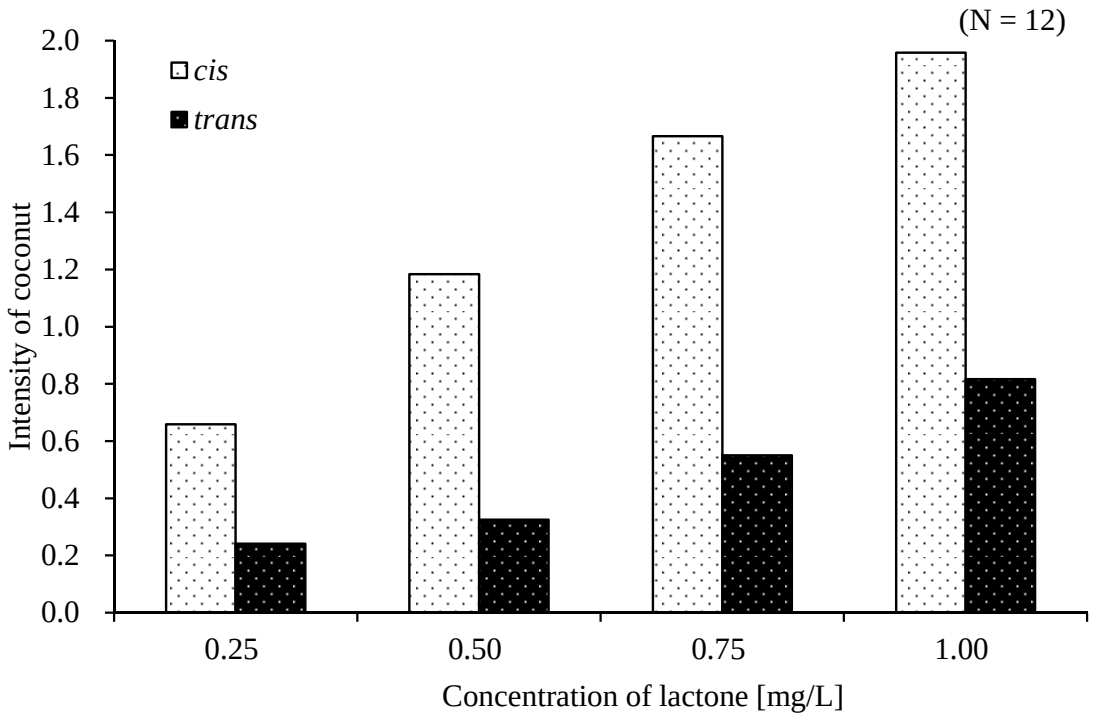


Figure 6.7 Intensity of coconut aroma in 20% abv ethanol solutions containing various amounts of either *cis* or *trans*-lactone

Significant differences were observed in the levels of 'coconut' aroma depending on the all concentrations of *cis*-lactones present ($p = <0.01$). In the case of *trans*-lactones, intensities (0.2, 0.3, 0.6, 0.8 respectively) were much less than those of *cis*-lactone (0.7, 1.2, 1.7, 2.0 respectively). However, the trend of gradual increase was observed which was same as *cis*-lactone, even though significant differences were not observed in the all concentrations ($p = 0.58, 0.13, 0.02$). As may have been expected, an increase in the levels of both *cis* and *trans*-lactone corresponded to an increase in aroma intensity. This indicated that amount of lactones used in these samples was at a suitable level for sensory discrimination. The intensity scores for *cis*-lactone were higher than those of the *trans*-lactone. This result was expected due to the lower sensory threshold of *cis*-lactone.

The intention was to apply this knowledge to further develop the prediction of aroma intensity using an equation. Intensity of a single aroma compound, I(compound) can

generally be expressed as a log first order polynomials function with a variable parameter, namely the level of the aroma compound present (Lawless et al., 1998). This function is shown as **F1**.

$$\mathrm{I(isomer)} = \ln(\mathrm{A[isomer]} + \mathrm{B})$$

where A and B are constants **(F1)**

Applying this function (**F1**) to the sensory data collected for the *cis*- and *trans*- isomers (**Figure 6.7**) gave rise to the formula shown in **F2** (**Table 6.9**) and **F3 (Table 6.10)**. Where A was determined to be the equation of the line plotted in **Figure 6.8** for *cis*-lactone and in **Figure 6.9** for *trans*-lactone. Approximations were calculated using the least squares method.

$$\mathrm{I}(\mathit{cis}) = \ln(5.6927 \text{ x } [\mathit{cis}] + 1) \quad \textbf{(F2)}$$

	Concentration of lactone [mg/L]			
	0.25	0.50	0.75	1.00
Intensity	0.7	1.2	1.7	2.0

Table 6.9 Approximations calculated using the least squares method for *cis*-lactone

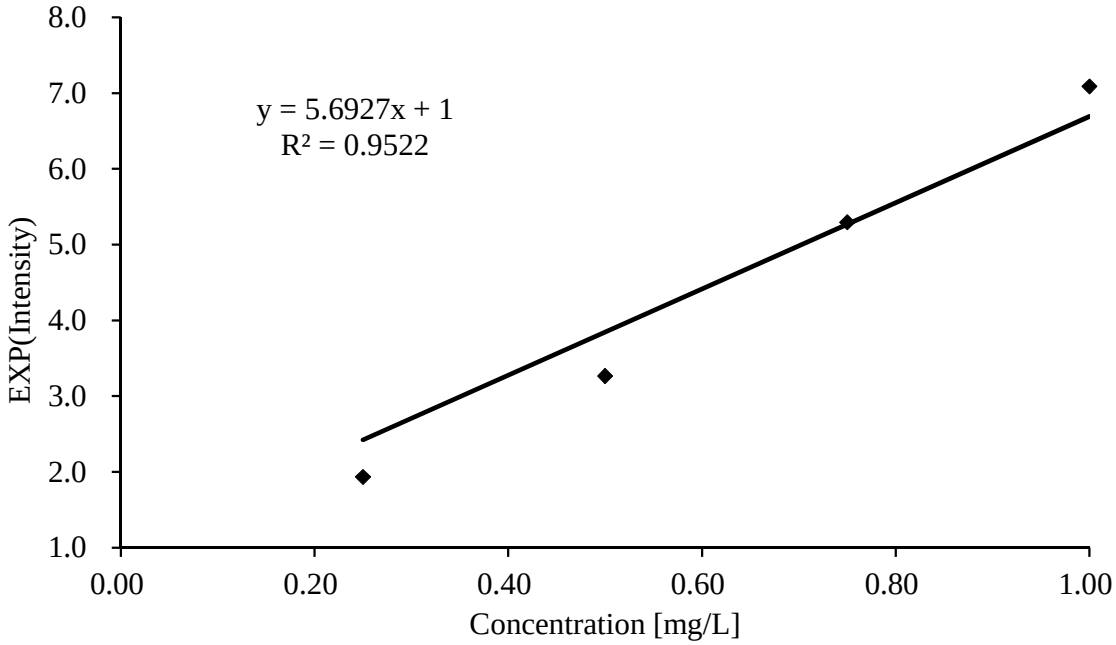


Figure 6.8 Approximations calculated using the least squares method for *cis*-lactone

$$\mathrm{I}(\mathit{trans}) = \ln(1.1057 \times [\mathit{trans}] + 1) \qquad \textbf{(F3)}$$

	Concentration of lactone [mg/L]			
	0.25	0.50	0.75	1.00
Intensity	0.2	0.3	0.6	0.8

Table 6.10 Intensity for approximations calculated using the least squares method for *trans*-lactone

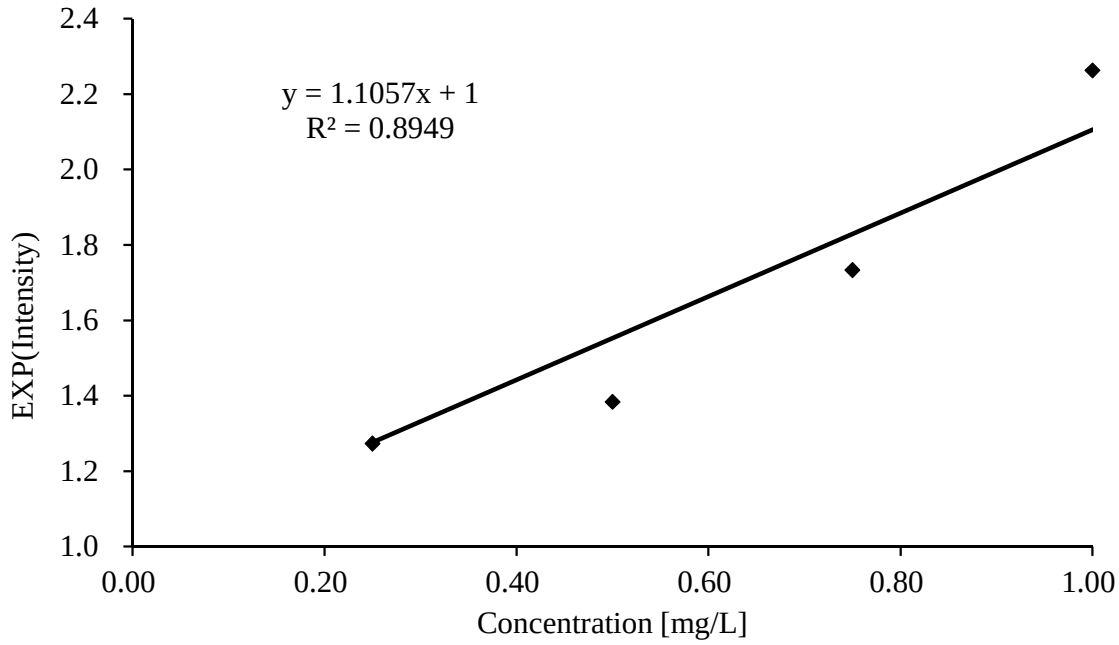


Figure 6.9 Approximations calculated using the least squares method for *trans*-lactone

Both formula (**F2** and **F3**) demonstrated a good linear trend, R^2 = 0.9522 and 0.8949 respectively, indicating that this function gave a good approximation of aroma intensity in the simple single isomer samples.

The intensities of both isomers at the determined detection thresholds of *cis*-lactone (0.15 mg/L) and *trans*-lactone (0.83 mg/L) (**Section 6.2.1**) were calculated using equations **F2** and **F3**. An approximated curve was calculated for the *cis*- and *trans*-lactones (**Figure 6.10**), and the intensities were determined to be I(*cis*-threshold) = 0.62 and I(*trans*-threshold) = 0.65. The intensities at the thresholds were both around 0.6 and therefore numerically very close to each other. From this result it can be estimated that the lowest intensity at which panellists can detect the presence of these lactones as contributing a coconut aroma is around 0.6.

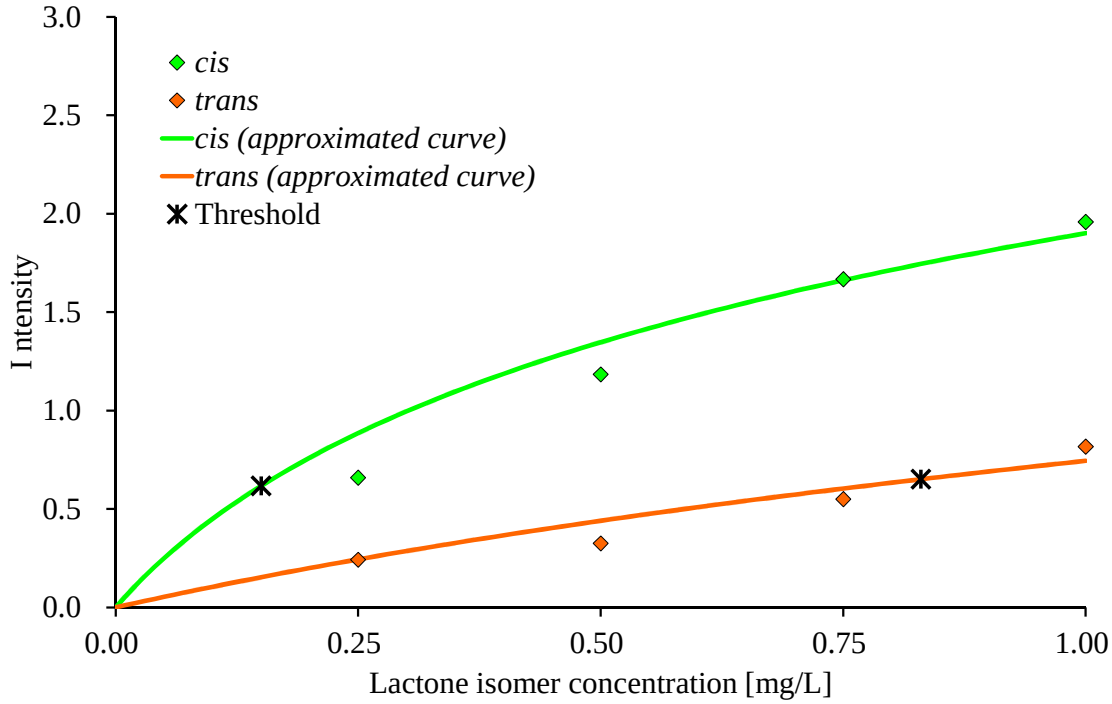


Figure 6.10 Approximated curve for intensities of coconut aroma in 20% abv ethanol solutions containing various amounts of either *cis* or *trans*-lactone

6.2.2.5.2 Sensory analysis of various ratio mixtures of isomers

Following on from the work with individual lactone isomers, further studies were undertaken using mixed solutions of the two lactone isomers in order to determine whether or not there were any synergistic sensory effects. The samples were prepared such that each contained a total of 1.00 mg/L lactone. The ratios of the two isomers were altered within the 1.00 mg/L addition of lactones.

The intensity of coconut aroma in these mixture systems underwent sensory analysis (**Section 2.4.1-3**) and the subsequent intensity scores are illustrated in **Figure 6.11**. When the isomers were assessed individually (**Table 6.8** and **Figure 6.7**), the *cis*-lactone was determined to have higher intensity scores than *trans*-lactone, therefore it was anticipated that in the mixture the *cis*-lactone would dominate. The results presented in

Figure 6.11 demonstrate that aroma intensity is not dominated by *cis*-lactone. The intensity scores increase before decreasing again, the peak intensity scores were around the 50:50 lactone ratio level. Furthermore, the intensity score for the 50:50 mix is 2.0. This is greater than the scores for given for either the *cis* or *trans*-lactones when present individually, which were 1.6 and 0.5 respectively.

		Concentration of lactone [mg/L]				
Lactone isomer	*cis*	0.00	0.25	0.50	0.75	1.00
	trans	1.00	0.75	0.50	0.25	0.00
Percentage of *cis* -lactone [%]		0	25	50	75	100
Mean		0.5	1.2	2.0	1.9	1.6
St. Dev.		0.5	0.5	0.6	0.6	0.8
p value by t-test (N = 10)		-	0.01	<0.01	<0.01	<0.01

Table 6.11 Intensity of coconut aroma in 20% abv ethanol solutions containing mixture of *cis* and *trans*-lactones

(Raw data is available in Appendix 13)

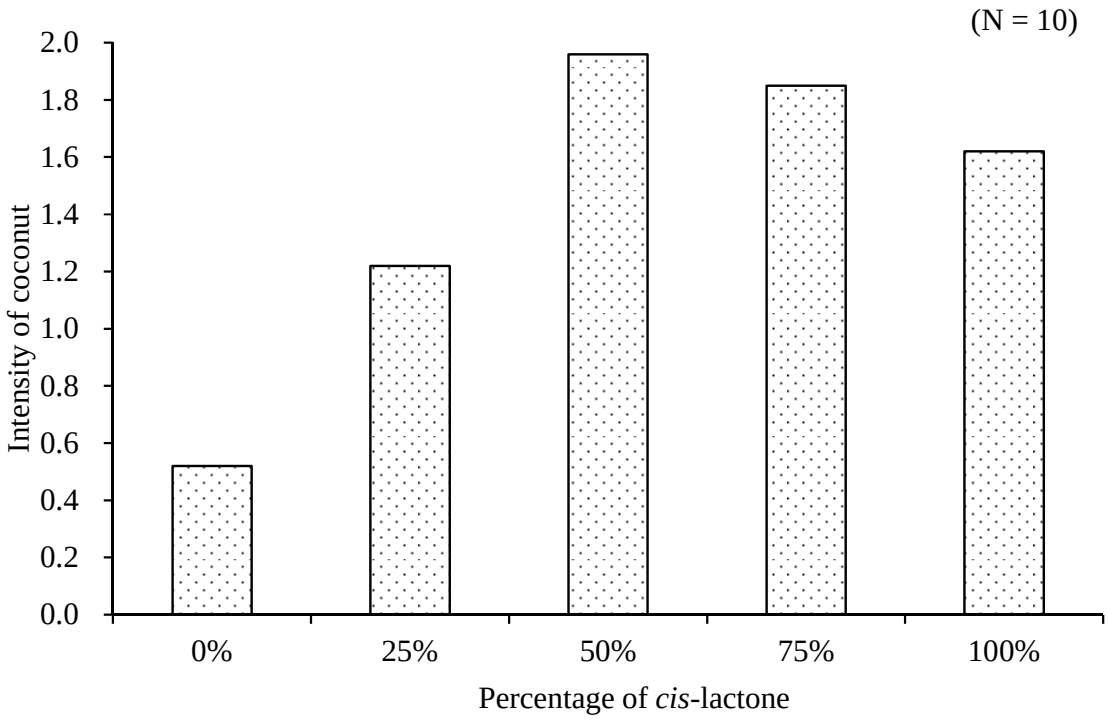


Figure 6.11 Intensity of coconut aroma in 20% abv ethanol solutions containing mixture of *cis* and *trans*-lactones

To confirm that this was synergistic effect, the calculated value, as described previously (**Section 6.2.2.3**) was applied to the data for the mixed isomer samples. The function **F1** (**Section 6.2.2.5.1**) was adapted to allow the levels of the two different isomers to be taken into account. The intensity of the mixture was initially calculated as a simple sum of both lactones. A log second order polynomials function was used, as given in **F4**.

$$\mathrm{I(mix)} = \ln(\mathrm{A}[\mathit{cis}] + \mathrm{B}[\mathit{trans}] + \mathrm{C})$$

where A, B and C are constants **(F4)**

The total amount of lactone in all of the samples was known to be 1.00 mg/L, which allowed the function **F4** to be simplified to a log first order polynomials function (**F5**) using only the concentration of *cis*-lactone as a variable. The resulting simplified function is similar to **F1**.

$$I(mix) = \ln(A[cis] + B[trans] + C)$$

$$= \ln((A - B)[cis] + (B + C))$$

$$= \ln(D[cis] + E)$$

Where D = A - B, and E = B + C are constants **(F5)**

This function was applied to the sensory data obtained for the mixed isomer samples (**Table 6.11**), the least squares method for approximation was used to calculate the 'intensity score' (**Table 6.12**, **Figure 6.12**). The slope of the **Figure 6.12** was applied to **F5**, producing equation **F6**.

		Concentration of lactone [mg/L]				
Lactone isomer	*cis*	0.00	0.25	0.50	0.75	1.00
	trans	1.00	0.75	0.50	0.25	0.00
Percentage of *cis* -lactone [%]		0	25	50	75	100
Intensity		0.5	1.2	2.0	1.9	1.6

Table 6.12 Approximations calculated using the least squares method for lactone mixture

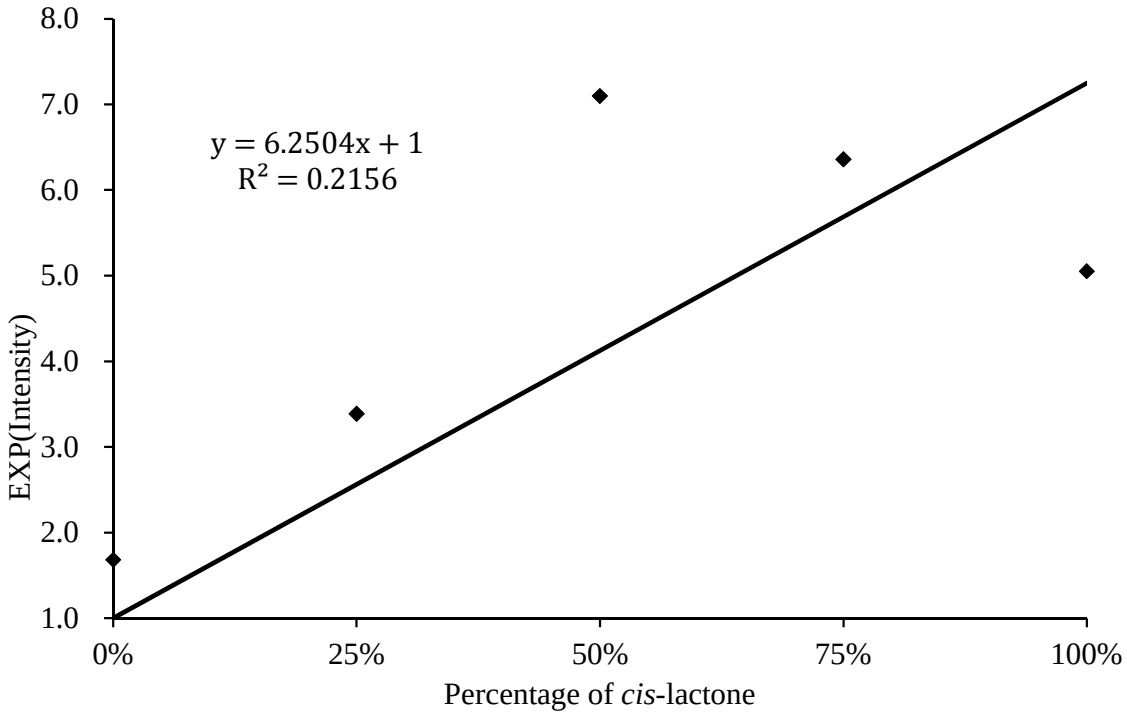


Figure 6.12 Approximations calculated using the least squares method for lactone mixture

$$I(\text{mix}) = \ln(6.2504 \text{ x } [cis] + 1) \qquad \textbf{(F6)}$$

The low R^2 value of 0.2156, (**Figure 6.12**) demonstrated that a function based on the simple additive impact of the two isomers did not provide a good approximation of the sensory data obtained for these mixtures. Therefore, an alternative formula was developed to take into account both synergistic and additive effects (**F7**). In order to express a synergistic effect, the combined terms of *cis* and *trans*-lactone were used.

$$I(\text{mix}) = \ln(A[cis] + B[trans] + C[cis]\,[trans] + D)$$

where A, B, C, and D are constants **(F7)**

As was previously outlined for **F5**, the function **F7** can be shown as a log second order polynomials function (**F8**) using only the concentration of *cis*-lactone as a variable.

$$I(mix) = \ln(A[cis] + B[trans] + C[cis]\,[trans] + D)$$

$$= \ln(-C[cis]^2 + (A - B + C)[cis] + (B + D))$$

$$= \ln(E[cis]^2 + F[cis] + G)$$

where E = – C, F = A – B + C, and G = B + D are constants

(F8)

Applying this function to the sensory data shown in **Table 6.11** and **Table 6.12** when plotted (**Figure 6.13**) gave the formula for the line which then resulted in the formula **F9**.

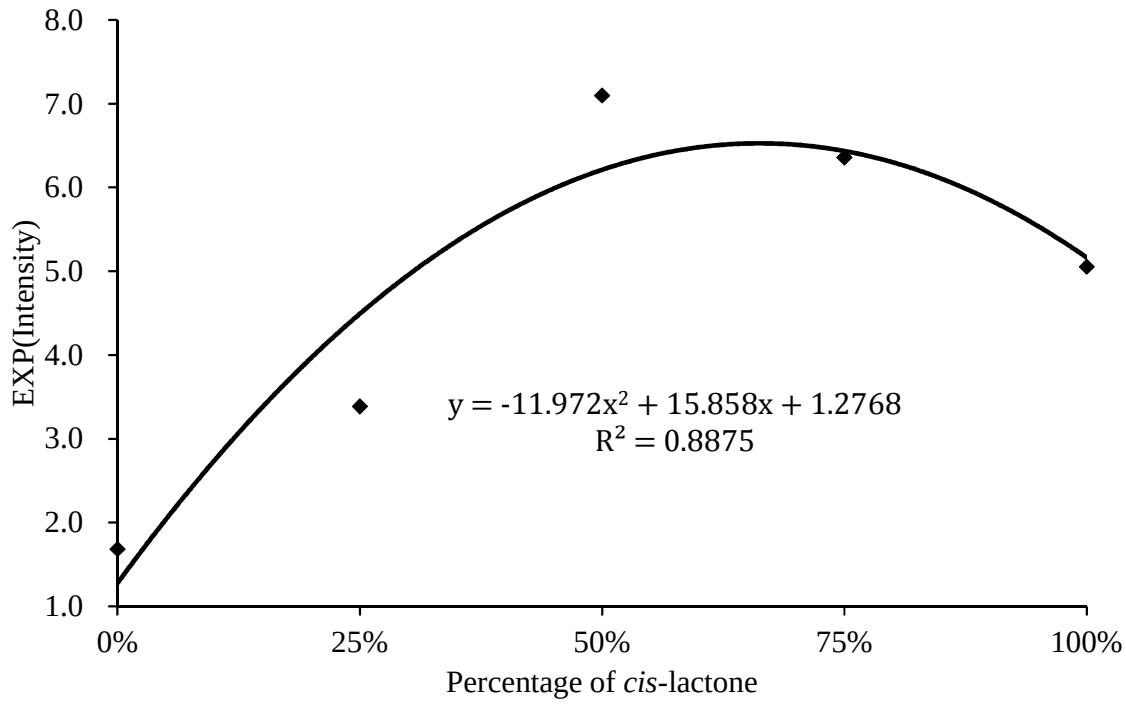


Figure 6.13 Approximations calculated using the least squares method for lactone mixture by second order polynomials function

$$I(mix) = \ln(-11.972 \times [cis]^2 + 15.858 \times [cis] + 1.2768)$$

$R^2 = 0.8875$ **(F9)**

The formula which was based on both synergistic and additive effects between the two isomers gave a greater R^2 (0.8875) value than the formula based on additive effects alone which had an R^2 of 0.2156.

In order to confirm the result predicted by **F9**, a hypothetical calculation of the intensity of mixed isomer solutions based on the data obtained for single isomers both presented in **Table 6.8** was performed. The intensity was calculated as a sum of isomers, approximated in the same form as **F4** or **F5**. The intensities were calculated and the results are presented I(mix') calculated was shown in **Table 6.13**. This data was plotted in **Figure 6.14** and the equation of the line used to develop function **F10** from function **F5**.

$$I(\text{mix}') = \ln(5.0172 \text{ x } [cis] + 2.1833)$$

$$R^2 = 0.9651 \qquad \textbf{(F10)}$$

		Concentration of lactone [mg/L]				
Lactone isomer	*cis*	0.00	0.25	0.50	0.75	1.00
	trans	1.00	0.75	0.50	0.25	0.00
Percentage of *cis* -lactone [%]		0	25	50	75	100
Intensity	*cis*	0.0	0.7	1.2	1.7	2.0
	trans	0.8	0.6	0.3	0.2	0.0
	total	0.8	1.3	1.5	1.9	2.0

Table 6.13 Approximations calculated using the least squares method for lactone mixture by sum of isomers

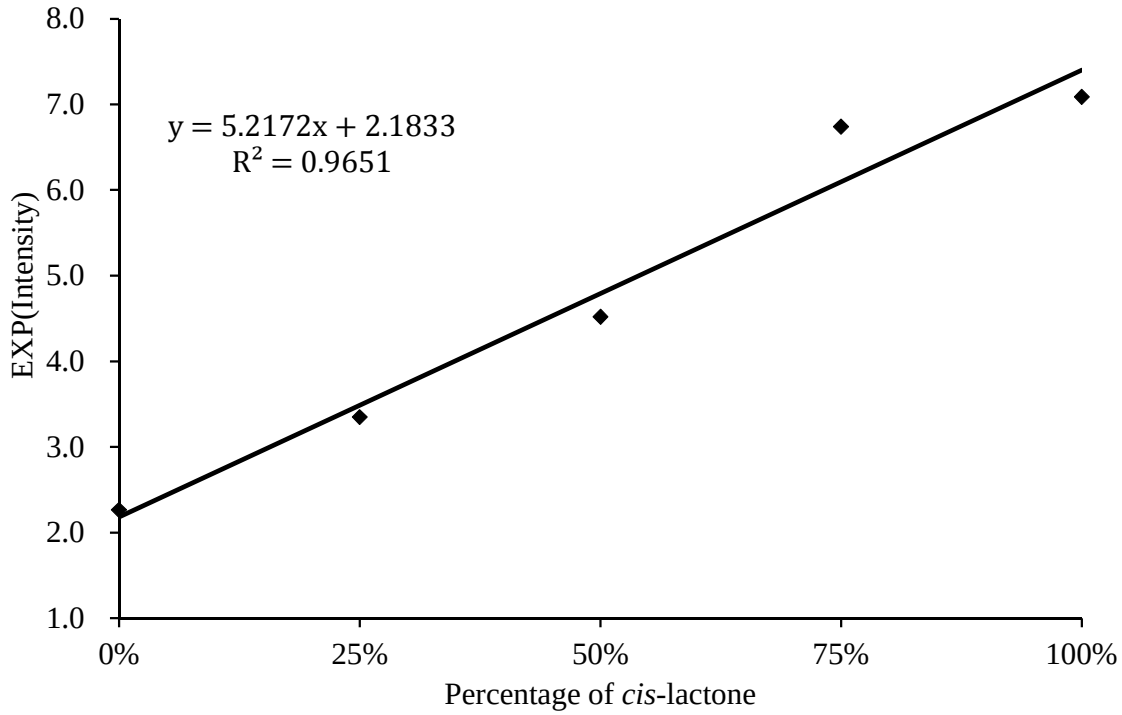
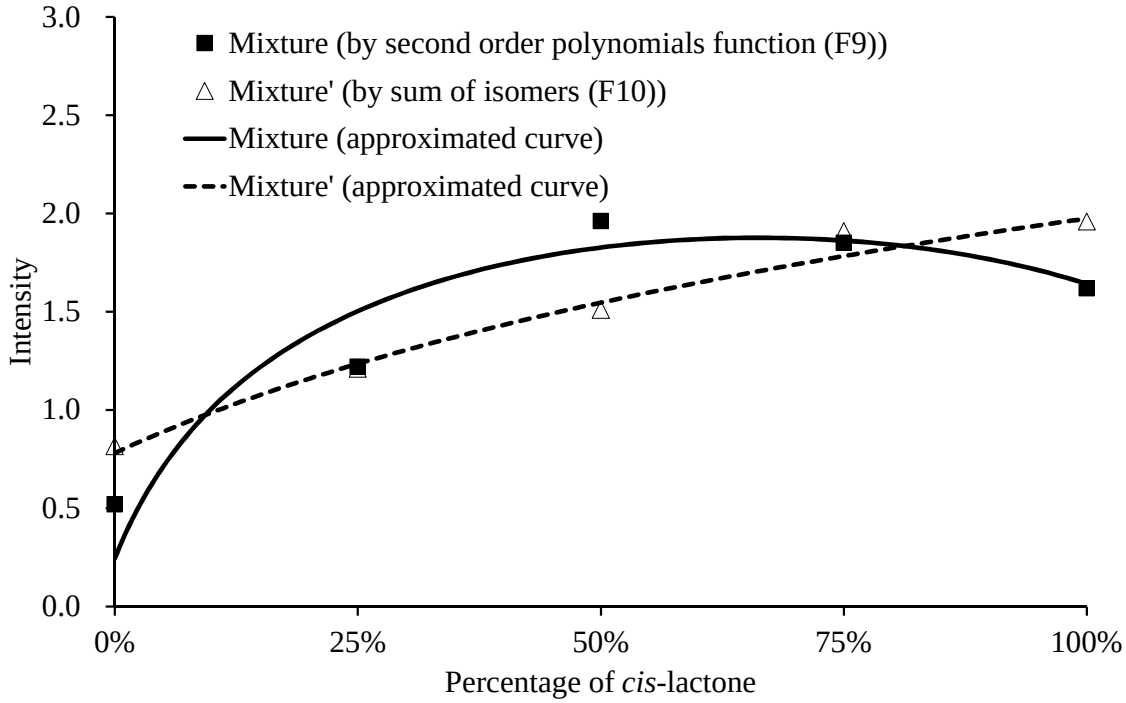


Figure 6.14 Approximations calculated using the least squares method for lactone mixture by sum of isomers

Comparison of the application of the data used to generate equations **F9** and **F10** is shown in **Figure 6.15**. These two curves display differing shapes, which suggests that intensity calculations by simple sums are different from the intensity of the actual mixed isomer samples. It is proposed that this confirms the existence of synergistic effects between *cis* and *trans*-lactones. In addition to this, the highest intensity is the same point as where the ratio *cis*:*trans* equals 2:1. Interestingly this figure is much closer to the ratio founded in JPN whisky than USA whisky (**Section 4.2.2**, **Table 4.4**). Therefore, the results of **F9** supports observation that JPN whisky has more coconut aroma than USA whisky. It is postulated that model equation (**F9**) can be used to predict the intensity of the coconut aroma of whisky using just the concentration of the *cis*-lactone isomer.



$$I(\text{mix}) = \ln(-11.972 \times [cis]2 + 15.858 \times [cis] + 1.2768) \quad \textbf{(F9)}$$

$$I(\text{mix}') = \ln(5.0172 \times [cis] + 2.1833) \quad \textbf{(F10)}$$

Figure 6.15 Comparison of intensity scores approximated from individual and mixed lactone data

6.2.3 Distinct aroma of whisky lactones

Although both *cis* and *trans*-lactones contribute to the coconut aroma, these are known to have subtly different aromas. *Cis*-lactone has been described as having a faint coconut note, faint musty, earthy note and is reminiscent of hay, whilst *trans*-lactone has a piquant celery note, faint coconut note and green walnut note (Koppenhoefer et al., 1994). The sensory panellists used during the course of this study were asked to describe these lactones using their own terminology (**Section 2.1.3**). This resulted in *cis*-lactone being described as 'heavy & oily' *trans*-lactone as 'light & fresh'. Sensory analysis was carried out using three coconut related descriptors; 'heavy & oily' coconut which is mainly derived from *cis*-lactone, 'light & fresh' coconut which is mainly derived from

trans-lactone, and 'total' coconut in order to investigate the coconut aroma interaction between the lactone isomers.

6.2.3.1 Study of coconut aromas in whiskies

Sensory analysis (**Section 2.4.1-3**) was performed on JPN and USA 20 year old whiskies, the sensory results are summarised in **Table 6.14** and illustrated in **Figure 6.16**. The JPN whisky (*cis*-lactone 0.41 mg/L, *trans*-lactone 0.82 mg/L) which contained the higher concentration of *trans*-lactone (**Section 4.2.2 Table 4.4**) was given the higher score of 0.9 for 'light & fresh' coconut aroma than USA whisky (*cis*-lactone 1.01 mg/L, *trans*-lactone 0.10 mg/L). Whereas for the USA whisky, the greatest lactone component was *cis*-lactone which was described as more 'heavy & oily'. When the JPN whisky was compared with USA whisky, all of sensory intensity scores (0.4, 0.3, and 0.4) were lower than those of the JPN whisky (1.0, 0.9, and 0.6). This was surprising as chemical analysis demonstrated that the USA whisky contained a higher concentration of the more aroma active *cis*-lactone.

	Amount [mg/L as is]			Total coconut	Light & fresh coconut	Heavy & oily coconut
	cis lactone	*trans* lactone				
JPN 20yo	0.41	0.82	Mean	1.0	0.9	0.6
			St. Dev.	0.8	0.8	0.6
USA 20yo	1.01	0.10	Mean	0.4	0.3	0.4
			St. Dev.	0.3	0.3	0.4
p value by t-test (N = 9)				0.09	0.09	0.54

Table 6.14 Sensory results of 20yo whiskies using three coconut related descriptors

(Raw data is available in Appendix 14)

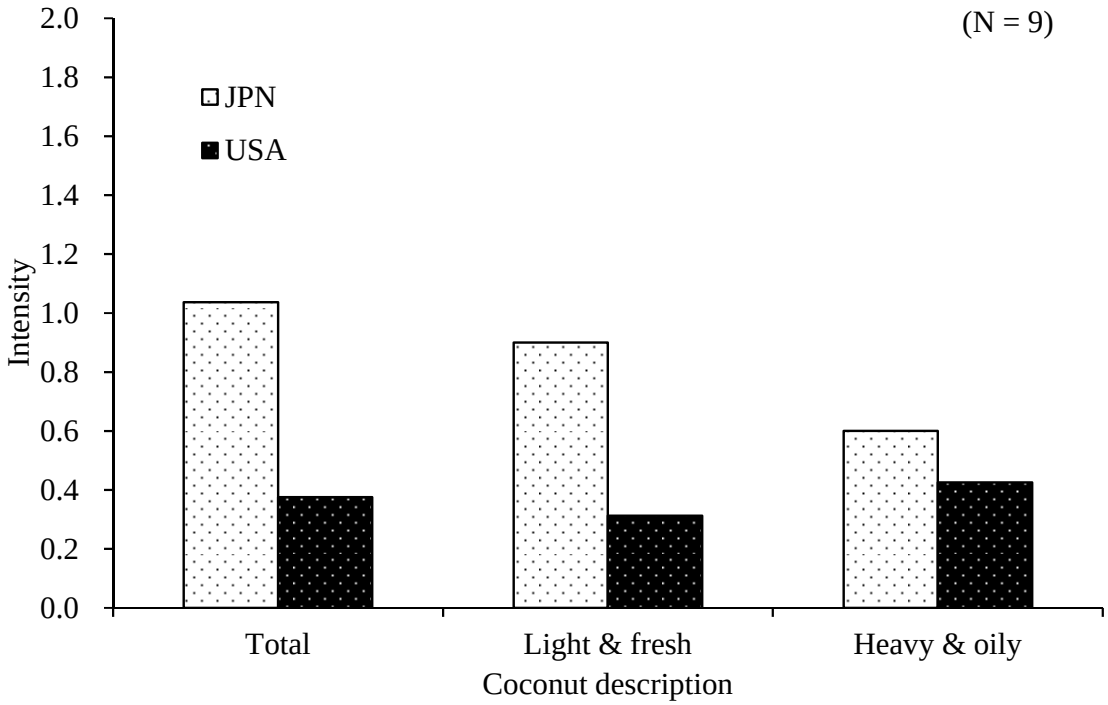


Figure 6.16 Sensory results of 20yo whiskies using three coconut related descriptors

6.2.3.2 Study of coconut aromas in model ethanol solutions

In order to clarify the relation between the lactone isomers and these three coconut related descriptors in whisky, ethanol solutions were prepared and sensory analysis was carried out.

The equations were tested through the use of two model solutions each containing one of the lactones (*cis*- or *trans*-). Lactones at a concentration of 1.00 mg/L in a 20% abv ethanol solution were used (**Section 2.1.5**). The sensory analysis was carried out using the three coconut related descriptors, 'heavy & oily' coconut, 'light & fresh' coconut and 'total' coconut (**Section 2.4.1-3**). The results from this sensory analysis are summarised in **Table 6.15** and illustrated in **Figure 6.17**.

When considering the *cis*-lactone solution, the score of 0.9 for 'light & fresh' was similar to the score of 1.0 for 'heavy & oily'. Whereas, considering the *trans*-lactone solution the score of 0.6 for 'light & fresh' was greater than the score of 0.2 for 'heavy & oily'. The intensity scores for the *cis*-lactone solution (1.3, 0.9, and 1.0) were all higher than those given to the *trans*-lactone solutions (0.5, 0.6, and 0.2) for all three coconut descriptors. It should be noted that the difference for 'light & fresh' was not significant ($p = 0.17$). This was not an unexpected result as *cis*-lactone is known to have a lower sensory threshold than *trans*-lactone. The 'heavy & oily' character was found to be dominant in *cis*-lactone, while *trans*-lactone was characterised by 'light & fresh' coconut notes. This agreed with the results from the sensory panel in the previous chapter (**Section 6.2.3.1**).

		Total coconut	Light & fresh coconut	Heavy & oily coconut
cis lactone	Mean	1.3	0.9	1.0
	St. Dev.	0.8	0.6	0.8
trans lactone	Mean	0.5	0.6	0.2
	St. Dev.	0.6	0.7	0.4
p value by t-test (N = 15)		<0.01	0.17	0.01

Table 6.15 Intensity of 20% abv ethanol model solutions including each lactone isomer using three coconut related descriptors

(Raw data is available in Appendix 15)

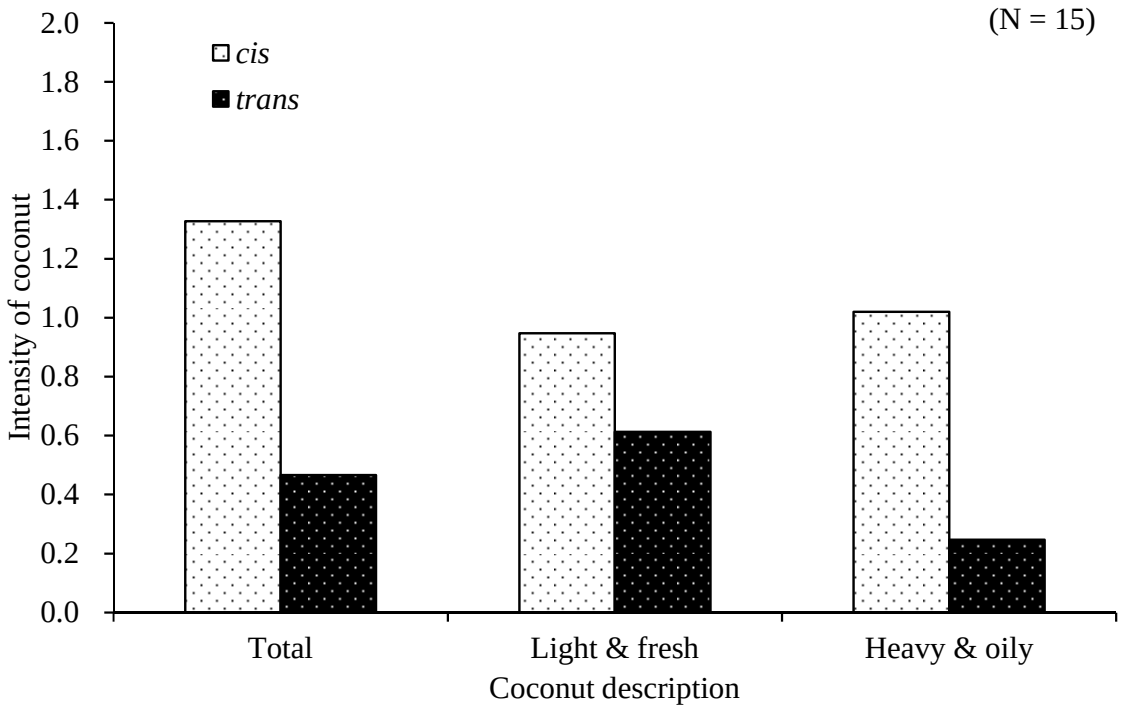


Figure 6.17 Intensity of 20% abv ethanol model solutions including each lactone isomer using three coconut related descriptors

6.2.3.3 Lactone addition experiments

The influence of each lactone in the whisky matrix was examined and explored in further detail with each lactone added to whisky. The addition experiments (**Section 2.1.6**) were carried out using both USA and JPN 20yo whiskies, which contained different original ratios of lactones (**Section 4.2.2**). An addition rate of 1.00 mg/L of each lactone was used, and the sensory analyses (**Section 2.4.1-3**) were carried out using three coconut related descriptors.

The sensory results obtained when each of the lactones were added to the 20 year old USA and JPN whiskies are summarised in **Table 6.16** and **Table 6.17** and illustrated in **Figure 6.18** (USA whisky) and **Figure 6.19** (JPN whisky) respectively.

	Amount [mg/L as is]			Total coconut	Light & fresh coconut	Heavy & oily coconut
	cis lactone	*trans* lactone				
No addition	1.01	0.10	Mean	0.4	0.3	0.4
			St. Dev.	0.3	0.2	0.3
cis addition	2.01	0.10	Mean	0.7	0.4	0.7
			St. Dev.	0.6	0.4	0.7
			p value by t-test (N = 8)	0.28	0.76	0.35
trans addition	1.01	1.10	Mean	1.1	0.8	0.7
			St. Dev.	0.6	0.4	0.8
			p value by t-test (N = 8)	0.02	0.02	0.36

Table 6.16 Sensory results of addition experiment to 20yo USA whisky using three coconut related descriptors

(Raw data is available in Appendix 16)

Sensory results obtained when each lactone isomer was added to the USA whisky (**Table 6.16** and **Figure 6.18**) demonstrated that when considering the no addition and *trans*-lactone addition, the scores for 'light & fresh' (0.3, 0.8) were similar to the scores for 'heavy & oily' (0.4, 0.7). Whereas the results for *cis*- addition found that the score of 0.4 for 'light & fresh' was less than the score of 0.7 for 'heavy & oily'. When *trans*-lactone was added, the intensity of 'light & fresh' coconut aroma increased from 0.3 to 0.8, while upon the addition of *cis*-lactone 'heavy & oily' increased from 0.4 to 0.7. This result was in line with what would be expected from the addition of these isomers and their associated sensory characteristics. The scores for 'total' coconut aroma were observed to increase from 0.4 to 1.1 when *trans*-lactone was added. This was surprising and suggests that, despite its lower sensory activity, this isomer has a stronger contribution to overall coconut aroma in USA whisky than the presence of *cis*-lactone.

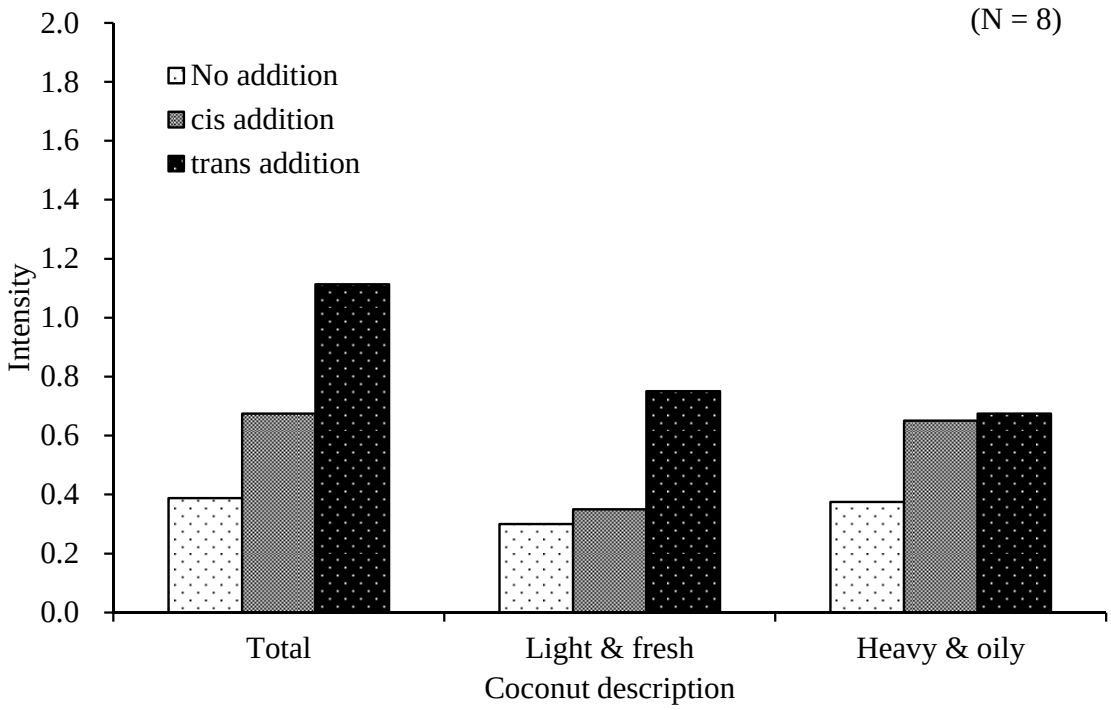


Figure 6.18 Sensory results of addition experiment to 20yo USA whisky using three coconut related descriptors

Sensory results obtained when the lactones were added to the JPN whisky (**Table 6.17** and **Figure 6.19**) demonstrated that the addition of *cis*-lactone had a positive impact on all descriptors, however the smallest effect was on 'light & fresh'. The addition of *trans*-lactone also had a positive impact on all three aroma descriptors with the greatest impact on the 'total' and 'light & fresh' characters. When *trans*-lactone was added, the intensity of 'light & fresh' coconut aroma increased from 0.6 to 1.0, while on *cis*-lactone addition 'heavy & oily' increased from 0.4 to the most 0.9. It suggested that when each lactone was added, the intensities of the typical characters associated with each lactone isomer increased. In both cases the 'total' coconut aroma also increased from 0.5 to 1.0 or 1.1. However, the scores were comparable for the two lactone isomers, indicating that in the JPN whisky *cis* and *trans*-lactone make a similar contribution to the 'total' coconut aroma.

	Amount [mg/L as is]			Total coconut	Light & fresh coconut	Heavy & oily coconut
	cis lactone	*trans* lactone				
No addition	0.41	0.82	Mean	0.5	0.6	0.4
			St. Dev.	0.3	0.5	0.3
cis addition	1.41	0.82	Mean	1.0	0.6	0.9
			St. Dev.	0.7	0.6	0.7
			p value by t-test (N = 13)	0.03	0.74	0.06
trans addition	0.41	1.82	Mean	1.1	1.0	0.6
			St. Dev.	0.8	0.7	0.5
			p value by t-test (N = 13)	0.02	0.06	0.27

Table 6.17 Sensory results of addition experiment to 20yo JPN whisky using three coconut related descriptors

(Raw data is available in Appendix 17)

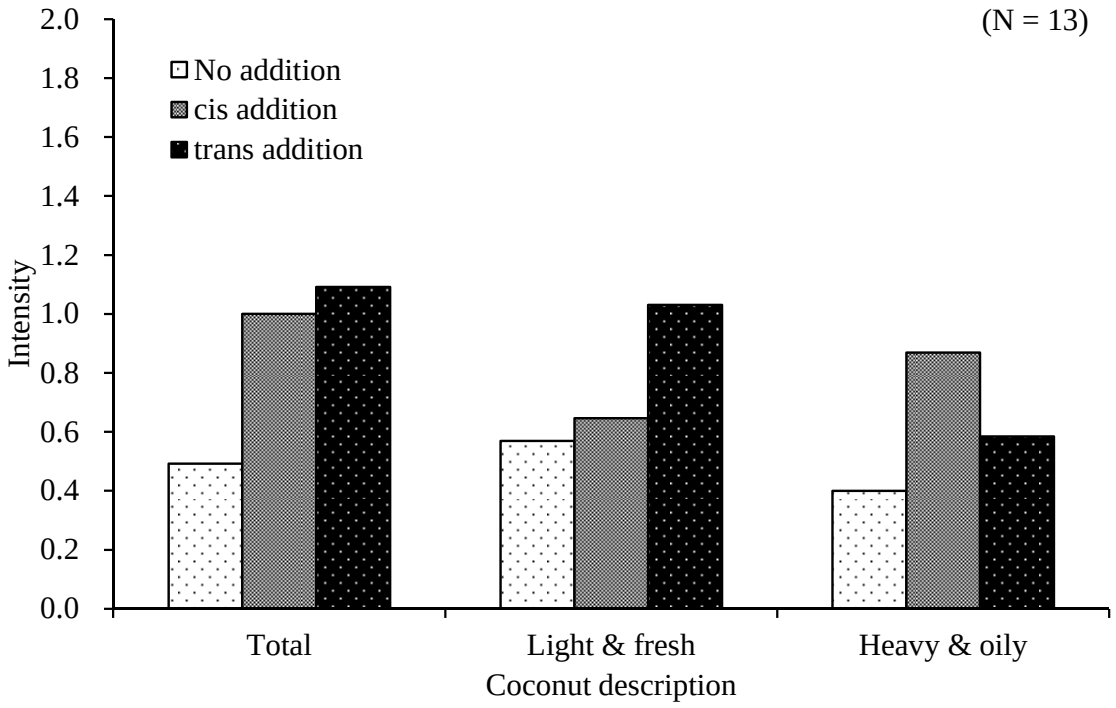


Figure 6.19 Sensory results of addition experiment to 20yo JPN whisky using three coconut related descriptors

6.3 Discussion

In order to measure the coconut aroma activity for each lactone, the threshold of *cis*-lactone, *trans*-lactone, and *cis* and *trans*-lactone 1:1 mixture in 20% abv ethanol were calculated (**Section 6.2.1**). The threshold of a 1:1 mixture of both lactones was 0.11 mg/L, lower than that of either *cis*-lactone (0.15 mg/L) or *trans*-lactone (0.83 mg/L) when these were measured individually. These results indicate that mixtures of lactones are more aroma active than when using *cis*-lactone or *trans*-lactone alone. It is postulated that there may be a synergistic effect between the two lactone isomers on the perception of coconut aroma by sensory panel. An equation was constructed by using the sum of coconut aroma intensity for each lactone isomer in order to predict coconut aroma based on a combination of the levels and thresholds of the two lactones (**Section 6.2.2.3**). Application of data to this equation found that the calculated value for predicted aroma intensity did not match the results of the sensory analysis. This added further weight to the proposed theory that there are synergistic effects. The addition of lactones to whisky was carried out in order to explore further these synergies (**Section 6.2.2.4**). *Trans*-lactone showed a highly active coconut aroma when added to whiskies containing different ratios of lactones. To enable study greater detail, model ethanol solutions were prepared which contained the levels of lactones found in whiskies (**Section 6.2.2.2**). This experimental protocol was used as coconut aroma is easier to detect in simple ethanol solutions than in a complex whisky matrix. Results for the model solutions demonstrated that the sample containing *trans*-lactone had a higher score than the sample containing no lactone and a similar score to the *cis*-lactone sample. These results led to the formation of two possible theories which are proposed here. The first is that the coconut aroma in whisky is mostly influenced by the ratio of lactones. The second is that *trans*-lactone is more aroma-active when also in the presence of *cis*-lactone. The

threshold results showed that the thresholds of 1:1 (w/w) mixture of both lactones was clearly lower than only *cis*-lactone or *trans*-lactone solutions (**Section 6.2.1**). The mixed lactones demonstrated greater aroma-activity than individual isomers. When taken together, the model sample behaviours and the threshold of mixed lactones together demonstrated a synergistic effect between the *cis*-lactone and *trans*-lactone, which may explain the high aroma activity of *trans*-lactone in whisky.

It was felt that it would be a useful tool for whisky blending if aroma intensity could be explained or, more accurately predicted, through the development of a numerical relationship (**Section 6.2.2.5.1**). The samples prepared all contained a total of 1.00 mg/L lactone, but with differing ratios of the two isomers, the aroma intensity was found not to be *cis*-lactone dominated, rather the intensity scores increase then decrease again peaking at around the 50:50 lactone ratio level (**Section 6.2.2.5.2**). Furthermore, the intensity score for the 50:50 is greater than the scores for given from either the *cis* or *trans*-lactones when present individually. When the expression of these aroma intensities as first or second order polynomials function was demonstrated, the second order polynomials function showed much more satisfactory fit (by R^2) than using a first order polynomials function. The second order polynomials function was based on both synergistic and additive effects between the two isomers. Therefore, the supposition of the existence of synergistic effects between *cis* and *trans*-lactone was supported by these results. This second order polynomials function pointed the highest intensity in the ratio *cis*:*trans* occurring at a ratio of 2:1. Interestingly this is much closer to the ratio found in JPN whisky than that of USA whisky. Therefore, it is suggested that the result of the occurrence of these lactone levels in whisky is that it corresponds to the observation that JPN whisky has more coconut aroma than USA whisky. As described previously, the

coconut aroma intensity can be predicted by the use of numerical calculation using the ratio of lactone isomers.

Finally, sensory analysis was carried out using three coconut related descriptors; 'heavy & oily' coconut which is mainly derived from *cis*-lactone, 'light & fresh' coconut which is mainly derived from *trans*-lactone, and 'total' coconut in order to investigate the coconut aroma interaction between the lactone isomers. As a result, the intensity scores for the *cis*-lactone solution were higher than those for *trans*-lactone for all three coconut descriptors, although the difference for 'light & fresh' was not significant (**Section 6.2.3.2**). This was not unexpected as *cis*-lactone is known to have a lower sensory threshold than *trans*-lactone. This suggests that *trans*-lactone can be detected as a 'light & fresh' aroma compound more readily than *cis*-lactone even though *trans*-lactone has higher threshold than *cis*-lactone. These results demonstrated that these three descriptors could be used by a trained sensory panel to recognise the aroma differences between the isomers.

In order to examine the influence of each lactone in the whisky matrix, each lactone was added to JPN and USA whiskies (**Section 6.2.3.3**). As a result, the intensities of the typical characters associated with each lactone isomer were increased, and the 'total' coconut aroma also increased in both cases by addition of either *cis*- or *trans*-lactone. These results indicated further the existence of a synergistic effect between the lactone isomers for the 'total' coconut aroma proposed in this chapter. The existence of a synergistic effect, in combination with the findings in this study, allows a number of conclusions to be proposed. The 'total' coconut aroma in the USA whisky was affected to a lesser degree by the addition of *cis*-lactone, which is the naturally occurring dominant lactone present in this spirit, than it is by the addition of *trans*-lactone. Whereas, in the

JPN whisky, which originally contained similar levels to each other of the two lactones, the levels were found to be very close to highest aroma activity (**Figure 6.15**). The 'total' coconut aroma was much less influenced by which isomer is added. Furthermore, when *trans*-lactone, which has higher threshold, was added the intensities in 'light & fresh' aroma, typical of *trans*-lactone, and 'total' coconut aroma both increased. Hence, the synergistic effect for 'total' coconut aroma is likely to be caused by the 'light & fresh' aroma from *trans*-lactone, whose aroma activity is enhanced by the interaction with *cis*-lactone.

Chapter 7: Conclusions & Future work

Since the whisky matured in Japanese oak cask has unique aromas which are not found in any other whisky matured in casks constructed of other oak types, the aim of this research was to identify the unique aromas imparted by casks of Japanese oak (**Section 1.5**).

'Incense' and 'Coconut' were identified as good expressions of the JPN whiskies (**Section 4.2.1**). When chemical analysis was focused on the coconut aroma compound, whisky lactone, only JPN whiskies were found have a different ratio of lactone isomers, weighted towards greater levels of *trans*-lactone (**Section 4.2.2**). In addition to this, it is indicated by the analysis of wood chip extraction (**Section 4.2.2**) that the greater levels of *trans*-lactone in JPN whiskies are very likely to be derived from the wood itself.

In order to determine the key factors involved in flavour activation, the wood chips using ethanol solution were analysed instead of cask maturation. The lactones and most of the aromatics were extracted consistently with no apparent relationship with depth of the visual whisky soaking observation (**Section 5.2.1.4**). In addition to this, it was found that the outside surface of the stave was not influenced by the maturation inside of the cask. When these chemical analyses of stave are applied for casks in production, this could be useful to predict how long or how many times the cask can be used as an active cask from wood extracts point of view. Therefore, a part of the company wood policy could be managed by the chemical analysis of stave instead of conventional sensory analysis.

When the effects of various heat treatments, which would be used normally for the regeneration of casks were studied, both the levels of lactones and their ratio which was an important aroma compound for the Japanese oak character in whisky were not activated by any heat treatments, although the regeneration of the colour and aromatics were partially shown (**Section 5.2.2.2**). Therefore, some aroma compounds such as vanillin are generated through heat treatment, while others, such as the lactones, cannot be regenerated once the levels are depleted by a period of maturation. From the production point of view, it was indicated that new casks are necessary to maintain consistent whisky aroma derived from wood because regeneration of cask by heat treatment is selective.

When sensory analyses of threshold measurements and lactone isomer addition to model solutions or whiskies were carried out, it is postulated that there may be the possibility of a synergetic effect between lactone isomers on the perception of coconut aroma (**Section 6.2.2.4**). In order to explain the effect, a numerical relationship was developed (**Section 6.2.2.5**). When the expression of the aroma intensities as first or second order polynomials function with lactone concentration was demonstrated, second order polynomials function which was based on both synergistic effects between the two isomers showed much more satisfactory fit than using a first order polynomials function (**Figure 6.15**). This second order polynomials function pointed the highest intensity in the ratio which is much closer to the ratio of JPN whisky than USA whisky. Therefore it is suggested that highly active coconut aroma of JPN whisky is derived from not only the concentration of lactone but also ratio of two isomers. If the concentrations of both lactones are analysed before whisky blending, this information could be useful to determine the blending recipe from the coconut aroma point of view. When the sensory analysis was carried out using coconut descriptors related to each lactone isomer, the

synergistic effect is likely to be caused by the aroma activity of *trans*-lactone enhanced by the interaction with *cis*-lactone (**Section 6.2.3**).

As described above, this study was focused on the coconut aroma and lactone isomers in JPN whisky. As a result, it could be demonstrated that only JPN whisky has a unique coconut aroma due to higher levels of *trans*-lactone which are never found in USA or SPA whisky. This means Japanese oak is an important raw material to appeal the uniqueness of Japanese whisky worldwide.

In this study, limited whiskies were used as samples for analysis and interesting results about the coconut character of Japanese whisky were obtained. In order to make this study more practical in industry, wider range of whiskies, maturation period, oak tree of locations or ages, and other factors, will be researched in the future. Additionally, further investigation of the change on coconut aroma, because the coconut aroma intensity of Japanese oak whisky was increased with time, but that of American oak whisky was decreased. In the meantime, whisky maturation normally needs at least a couple of years and especially for Japanese oak whisky, more than 20 years is needed until being unique. Therefore already matured whiskies were used as samples for analysis. In order to ascertain the result of this study, new maturation trial will be started and traced using long period. Furthermore, ‘incense’ was determined as an alternative important character of Japanese oak whisky in addition to coconut character. As the compound of ‘incense’ or other unique characters of Japanese oak whisky have not been studied, the identification of fascinating Japanese oak characters will be studied in order to demonstrate the uniqueness of Japanese whisky.

Appendix

Appendix 1

Sensory results of 20 year old JPN and USA whiskies using the aroma descriptions of incense, matured pineapple, and coconut

		Incense	Matured pineapple	Coconut
	JPN-20yo	0.8	0.6	0.9
		0.9	0.8	1.3
		2.0	0.5	1.5
		2.0	0.0	1.0
		0.3	0.1	0.6
		0.5	1.0	0.5
		1.7	1.0	2.5
		2.0	1.5	2.0
		2.0	1.8	2.2
Mean		1.4	0.8	1.4
St. Dev.		0.7	0.6	0.7
	USA-20yo	1.0	0.7	0.6
		0.5	0.2	0.5
		0.5	1.0	1.0
		0.5	0.0	0.0
		1.9	0.1	1.7
		1.0	0.5	1.0
		2.0	1.5	1.0
		0.5	0.5	1.0
		1.4	0.5	0.6
Mean		1.0	0.6	0.8
St. Dev.		0.6	0.4	0.4
p value by t-test (N = 9)		0.40	0.26	0.09

Appendix 2

Sensory results of 27 year old JPN and USA whiskies using the aroma descriptions of incense, matured pineapple, and coconut

		Incense	Matured pineapple	Coconut
	JPN-27yo	1.2	0.3	0.8
		2.1	2.0	1.4
		1.0	0.5	1.0
		0.5	2.5	0.5
		2.0	0.1	0.8
		1.5	0.7	0.5
		2.5	1.2	0.8
		2.5	2.0	2.5
		2.9	2.0	0.2
Mean		1.8	1.3	0.9
St. Dev.		0.8	0.8	0.6
	USA-27yo	0.7	0.6	0.5
		1.4	0.9	0.6
		0.5	0.0	1.0
		0.5	1.5	0.0
		0.9	0.1	0.3
		1.0	1.0	0.5
		1.5	1.2	0.5
		0.5	1.0	1.0
		2.7	2.5	0.5
Mean		1.1	1.0	0.5
St. Dev.		0.7	0.7	0.3
p value by t-test (N = 9)		0.01	0.22	0.05

Appendix 3

Sensory results of 40 year old JPN and USA whiskies using the aroma descriptions of incense, matured pineapple, and coconut

		Incense	Matured pineapple	Coconut
	JPN-40yo	0.8	0.2	2.9
		1.8	1.6	1.2
		3.0	2.0	0.5
		1.5	0.0	1.0
		2.9	0.1	1.0
		2.0	1.5	1.0
		1.8	0.1	2.8
		3.0	3.0	3.0
		1.8	0.9	2.9
Mean		2.1	1.0	1.8
St. Dev.		0.7	1.0	1.0
	USA-40yo	1.1	0.2	0.4
		0.7	0.5	0.7
		1.0	2.0	1.0
		0.5	0.0	0.0
		1.4	0.1	0.2
		1.5	0.8	1.0
		0.5	1.0	0.3
		0.5	1.0	0.5
		2.3	1.9	0.9
Mean		1.1	0.8	0.6
St. Dev.		0.6	0.7	0.3
p value by t-test (N = 9)		0.02	0.52	0.01

Appendix 4

Effect of extraction length of wood chip on subsequent extraction of lactones into 60% abv solution

		cis lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* +*trans*) [mg/L as is]	Ratio (*cis* /*trans*)
	1day	4.84	4.82	9.66	1.01
		5.50	5.41	10.91	1.02
		5.78	5.65	11.43	1.02
		5.65	5.53	11.18	1.02
Mean		5.44	5.35	10.80	1.02
St. Dev.		0.4	0.3	0.7	0.0
	2days	5.07	4.73	9.81	1.07
		5.20	5.10	10.30	1.02
		4.95	4.93	9.88	1.00
		5.06	5.07	10.13	1.00
Mean		5.07	4.96	10.03	1.02
St. Dev.		0.1	0.1	0.2	0.0
	7days	5.28	4.96	10.25	1.06
		5.29	5.13	10.42	1.03
		5.19	5.08	10.26	1.02
		5.45	5.33	10.78	1.02
Mean		5.30	5.13	10.43	1.03
St. Dev.		0.1	0.1	0.2	0.0
	30days	5.44	5.21	10.66	1.04
		5.27	5.12	10.39	1.03
		5.25	5.11	10.35	1.03
		5.44	5.39	10.83	1.01
Mean		5.35	5.21	10.56	1.03
St. Dev.		0.1	0.1	0.2	0.0
p value by ANOVA (N = 4)		0.18	0.15	0.15	0.70

Appendix 5

Effect of extraction alcohol strength of wood chip on subsequent extraction of lactones into ethanol solution

		cis lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
	20% abv	3.11	2.95	6.06	1.06
		3.06	3.24	6.30	0.94
		3.44	3.33	6.76	1.03
		3.80	3.78	7.58	1.00
Mean		3.35	3.33	6.67	1.01
St. Dev.		0.3	0.3	0.6	0.0
	40% abv	4.79	4.52	9.30	1.06
		5.15	4.94	10.09	1.04
		6.37	6.09	12.45	1.05
		5.37	5.24	10.61	1.02
Mean		5.42	5.20	10.62	1.04
St. Dev.		0.6	0.6	1.2	0.0
	60% abv	4.84	4.82	9.66	1.01
		5.50	5.41	10.91	1.02
		5.78	5.65	11.43	1.02
		5.65	5.53	11.18	1.02
Mean		5.44	5.35	10.80	1.02
St. Dev.		0.4	0.3	0.7	0.0
	80% abv	5.75	5.64	11.38	1.02
		6.05	5.99	12.04	1.01
		5.73	5.65	11.38	1.01
		5.64	5.62	11.26	1.00
Mean		5.79	5.72	11.51	1.01
St. Dev.		0.2	0.2	0.3	0.0
p value by ANOVA (N = 4)		<0.01	<0.01	<0.01	0.28

Appendix 6

Chemical analysis result of whisky lactones in young whiskies

		Strength [% abv]	*cis* lactone [mg/L as is]	*trans* lactone [mg/L as is]	Total (*cis* + *trans*) [mg/L as is]	Ratio (*cis* / *trans*)
	No treatment	56.6	0.60	0.70	1.31	0.86
		56.0	0.56	0.97	1.53	0.57
		57.4	0.66	1.75	2.40	0.38
		58.8	0.72	1.57	2.29	0.46
		59.7	0.63	1.01	1.63	0.63
		59.8	0.54	0.77	1.31	0.70
		59.4	0.68	0.84	1.53	0.81
Mean			0.63	1.09	1.71	0.58
St. Dev.			0.06	0.38	0.42	0.16
	Re-charring	56.8	0.72	1.20	1.92	0.60
		56.3	0.87	1.08	1.94	0.81
		57.1	0.73	1.27	2.00	0.58
		59.4	0.82	1.50	2.32	0.55
		59.1	0.59	0.79	1.38	0.75
		59.8	0.87	1.65	2.51	0.53
		58.4	0.55	1.28	1.84	0.43
Mean			0.74	1.25	1.99	0.59
St. Dev.			0.12	0.26	0.34	0.12
p value by t-test (N = 7)			0.31	0.11	0.24	0.44

Appendix 7

Chemical analysis result of aromatics in young whiskies

		Strength [% abv]	Vanillic acid [mg/L as is]	Vanillin [mg/L as is]	Syringic acid [mg/L as is]	Syringaldehyde [mg/L as is]	Sinapaldehyde [mg/L as is]
	No treatment	56.6	2.16	3.29	1.83	5.00	0.56
		56.0	2.43	3.80	1.80	5.12	0.50
		57.4	1.97	3.18	1.88	4.72	0.62
		58.8	1.92	2.78	2.04	4.38	0.47
		59.7	1.72	2.70	1.47	3.83	0.80
		59.8	1.55	2.45	1.49	3.31	0.89
		59.4	1.60	2.50	1.38	3.50	0.56
Mean			1.91	2.96	1.70	4.27	0.63
St. Dev.			0.29	0.45	0.23	0.67	0.14
	Re-charring	56.8	2.73	4.42	2.96	7.52	0.68
		56.3	1.93	3.41	2.07	6.06	0.87
		57.1	2.13	3.42	2.35	5.65	0.81
		59.4	1.80	3.08	2.29	5.56	0.87
		59.1	2.48	4.32	2.34	6.43	0.78
		59.8	1.97	3.48	2.78	6.69	1.13
		58.4	1.99	3.34	2.37	5.90	0.72
Mean			2.15	3.64	2.45	6.26	0.84
St. Dev.			0.31	0.48	0.28	0.64	0.14
p value by t-test (N = 7)			0.18	0.05	<0.01	<0.01	<0.01

Appendix 8

Sensory results of addition experiment to USA 20yo whisky using coconut descriptor

		No addition	*cis* addition	*trans* addition
	USA-20yo	0.3	0.2	0.1
		0.2	0.2	0.2
		0.8	1.0	0.9
		0.5	0.5	0.8
		1.0	0.5	0.5
		0.0	1.5	1.5
		0.4	0.7	0.2
		0.5	2.0	1.0
		0.0	0.0	1.0
		0.3	0.2	0.6
		0.0	0.5	0.8
		0.5	1.0	2.5
		0.6	0.2	1.7
		1.0	1.6	1.3
Mean		0.4	0.7	0.9
St. Dev.		0.3	0.6	0.6
p value by t-test (N = 14)			0.14	0.02

Appendix 9

Sensory results of addition experiment to JPN 20yo whisky using coconut descriptor

		No addition	*cis* addition	*trans* addition
	JPN-20yo	0.4	0.2	0.5
		0.8	0.7	1.2
		0.9	1.2	0.0
		0.5	1.7	1.3
		0.0	2.0	2.0
		0.5	0.2	0.0
		0.5	1.5	1.0
		0.0	1.0	1.5
		0.4	0.3	0.6
		0.0	0.5	0.3
		1.0	2.0	2.5
		0.7	0.1	2.1
		0.7	1.6	1.2
Mean		0.5	1.0	1.1
St. Dev.		0.3	0.7	0.8
p value by t-test (N = 13)			0.03	0.02

Appendix 10

Intensity of coconut aroma in samples of 20% abv ethanol model solutions containing each lactone

	No addition	*cis* lactone	*trans* lactone
	0.1	0.7	0.1
	0.3	0.3	0.2
	1.1	1.4	1.1
	0.0	1.5	0.0
	0.7	1.7	0.5
	0.0	3.0	0.0
	0.0	0.4	0.0
	0.0	2.0	0.0
	0.0	1.0	1.5
	0.0	1.0	0.0
	0.0	0.0	0.0
	0.5	1.5	0.5
	0.0	3.0	2.0
	0.8	1.5	0.6
	0.5	0.9	0.5
Mean	0.3	1.3	0.5
St. Dev.	0.4	0.8	0.6
p value by t-test (N = 15)		<0.01	0.29

Appendix 11

Intensity of coconut aroma in samples of 20% abv ethanol model solutions on replicating of JPN or USA 20yo whisky

		No addition	*cis* addition	*trans* addition
	JPN-20yo model solution	0.5	0.7	0.7
		1.0	1.8	1.9
		1.2	2.0	1.7
		1.0	2.3	1.7
		1.0	2.0	1.0
		0.7	1.2	1.0
		0.2	0.4	0.3
		1.3	1.0	1.8
		1.0	3.0	3.0
Mean		0.9	1.6	1.5
St. Dev.		0.3	0.8	0.8
p value by t-test (N = 9)			0.04	0.07
	USA-20yo model solution	0.4	0.7	0.3
		2.0	1.7	1.5
		1.4	1.4	1.0
		0.8	2.1	1.6
		2.0	1.0	2.5
		1.7	2.1	1.8
		0.5	1.0	1.0
		0.3	0.5	1.0
		0.5	2.0	1.5
		0.5	2.0	3.0
Mean		1.0	1.5	1.5
St. Dev.		0.7	0.6	0.7
p value by t-test (N = 10)			0.15	0.14

Appendix 12

Intensity of coconut aroma in 20% abv ethanol solutions containing various amounts of either *cis* or *trans*-lactone

		Concentration of lactone [mg/L]			
		0.25	0.50	0.75	1.00
	cis lactone	0.6	1.1	2.1	2.6
		0.5	1.3	1.0	1.6
		1.1	2.0	1.8	2.3
		0.3	1.0	1.5	2.2
		0.5	0.5	2.0	1.0
		1.5	1.9	2.3	2.6
		1.0	1.5	2.5	2.0
		0.5	1.2	0.7	1.7
		1.0	1.2	1.6	1.7
		0.2	0.5	1.0	1.3
		0.5	1.0	1.5	2.0
		0.2	1.0	2.0	2.5
Mean		0.7	1.2	1.7	2.0
St. Dev.		0.4	0.4	0.5	0.5
p value by t-test (N = 12)		-	<0.01	<0.01	<0.01
	trans lactone	0.0	0.0	0.2	0.2
		0.0	0.0	0.0	0.0
		0.5	0.5	0.0	0.5
		0.1	0.1	0.2	0.2
		0.0	0.2	1.2	1.5
		0.5	0.0	0.0	1.0
		0.1	0.2	0.3	0.5
		1.0	1.5	2.0	2.5
		0.0	0.1	0.3	0.4
		0.3	0.5	0.9	1.2
		0.3	0.3	0.5	0.3
		0.1	0.5	1.0	1.5
Mean		0.2	0.3	0.6	0.8
St. Dev.		0.3	0.4	0.6	0.7
p value by t-test (N = 12)		-	0.58	0.13	0.02

Appendix 13

Intensity of coconut aroma in 20% abv ethanol solutions containing mixture of *cis* and *trans*-lactones

		Concentration of lactone [mg/L]				
Lactone isomer	*cis*	0.00	0.25	0.50	0.75	1.00
	trans	1.00	0.75	0.50	0.25	0.00
Percentage of *cis* -lactone [%]		0	25	50	75	100
		0.5	1.5	3.0	2.0	2.0
		0.0	1.0	0.9	0.2	0.3
		0.8	1.1	2.1	1.8	1.5
		1.2	0.8	2.1	2.1	2.0
		1.5	1.9	2.5	2.6	2.3
		0.5	2.0	2.0	2.0	0.0
		0.2	1.4	1.3	1.6	1.9
		0.0	0.3	2.1	1.8	1.2
		0.0	0.5	1.5	2.0	2.5
		0.5	1.7	2.1	2.4	2.5
Mean		0.5	1.2	2.0	1.9	1.6
St. Dev.		0.5	0.5	0.6	0.6	0.8
p value by t-test (N = 10)		-	0.01	<0.01	<0.01	<0.01

Appendix 14

Sensory result of 20yo whiskies using three coconut related descriptors

		Total coconut	Light & fresh coconut	Heavy & oily coconut
	JPN-20yo	0.1	0.2	0.5
		0.8	0.6	0.4
		1.3	0.4	0.9
		0.5	1.0	0.5
		1.0	1.0	0.0
		0.0	0.0	0.0
		2.5	2.5	2.0
		2.1	1.5	0.5
Mean		1.0	0.9	0.6
St. Dev.		0.8	0.8	0.6
	USA-20yo	0.1	0.2	0.3
		1.1	1.0	0.5
		0.5	0.0	0.2
		0.5	0.5	1.5
		0.0	0.0	0.0
		0.0	0.0	0.0
		0.5	0.5	0.5
		0.3	0.3	0.4
Mean		0.4	0.3	0.4
St. Dev.		0.3	0.3	0.4
p value by t-test (N = 9)		0.09	0.09	0.54

Appendix 15

Intensity of 20% abv ethanol model solutions including each lactone isomer using three coconut related descriptors

		Total coconut	Light & fresh coconut	Heavy & oily coconut
	cis lactone	0.7	0.8	0.3
		0.3	0.5	0.3
		1.4	1.1	1.8
		1.5	0.3	1.5
		1.7	1.7	1.1
		3.0	1.0	2.0
		0.4	0.5	0.1
		2.0	2.0	1.0
		1.0	1.0	0.0
		1.0	0.9	1.6
		0.0	0.1	0.2
		1.5	0.3	1.2
		3.0	1.0	3.0
		1.5	1.0	0.2
		0.9	2.0	1.0
Mean		1.3	0.9	1.0
St. Dev.		0.8	0.6	0.8
	trans lactone	0.1	0.2	0.1
		0.2	0.2	0.1
		1.1	0.8	0.5
		0.0	0.0	0.0
		0.5	1.0	0.1
		0.0	0.0	0.0
		0.0	0.0	0.0
		0.0	0.5	0.0
		1.5	1.5	0.0
		0.0	0.0	0.0
		0.0	0.0	0.3
		0.5	0.5	0.0
		2.0	2.5	1.5
		0.6	0.8	0.1
		0.5	1.2	1.0
Mean		0.5	0.6	0.2
St. Dev.		0.6	0.7	0.4
p value by t-test (N = 15)		<0.01	0.17	0.01

Appendix 16

Sensory results of addition experiment to 20yo USA whisky using three coconut related descriptors

		Total coconut	Light & fresh coconut	Heavy & oily coconut
	No addition	0.2	0.1	0.1
		0.8	0.4	0.8
		0.5	0.3	0.2
		0.5	0.5	1.0
		0.0	0.0	0.0
		0.0	0.0	0.0
		0.5	0.5	0.5
		0.6	0.6	0.4
Mean		0.4	0.3	0.4
St. Dev.		0.3	0.2	0.3
	cis addition	0.2	0.1	0.2
		1.0	0.8	1.2
		0.5	0.3	0.2
		2.0	0.5	2.0
		0.0	0.0	0.0
		0.5	0.0	0.5
		1.0	1.0	1.0
		0.2	0.1	0.1
Mean		0.7	0.4	0.7
St. Dev.		0.6	0.4	0.7
p value by t-test (N = 8)		0.28	0.76	0.35
	trans addition	0.2	0.2	0.2
		0.9	0.6	1.1
		0.8	0.8	0.2
		1.0	1.0	0.0
		1.0	0.5	0.5
		0.8	0.5	0.3
		2.5	1.5	2.5
		1.7	0.9	0.6
Mean		1.1	0.8	0.7
St. Dev.		0.6	0.4	0.8
p value by t-test (N = 8)		0.02	0.02	0.36

Appendix 17

Sensory results of addition experiment to 20yo JPN whisky using three coconut related

		Total coconut	Light & fresh coconut	Heavy & oily coconut
	No addition	0.4	0.3	0.4
		0.8	1.1	0.6
		0.9	0.9	0.8
		0.5	0.5	0.5
		0.0	0.0	0.0
		0.5	0.5	0.5
		0.5	0.5	0.0
		0.0	0.0	0.0
		0.4	0.3	0.4
		0.0	0.0	0.0
		1.0	1.5	1.0
		0.7	0.5	0.3
		0.7	1.3	0.7
Mean		0.5	0.6	0.4
St. Dev.		0.3	0.5	0.3
	cis addition	0.2	0.2	0.2
		0.7	1.1	0.9
		1.2	1.8	0.0
		1.7	0.8	1.8
		2.0	0.0	1.5
		0.2	0.2	0.5
		1.5	0.0	1.5
		1.0	1.0	0.0
		0.3	0.4	0.2
		0.5	0.0	0.5
		2.0	2.0	2.5
		0.1	0.2	0.5
		1.6	0.7	1.2
Mean		1.0	0.6	0.9
St. Dev.		0.7	0.6	0.7
p value by t-test (N = 13)		0.03	0.74	0.06
	trans addition	0.5	0.5	0.2
		1.2	1.4	0.7
		0.0	0.2	0.0
		1.3	1.5	1.2
		2.0	1.5	0.0
		0.0	0.5	0.5
		1.0	1.5	0.5
		1.5	1.0	0.5
		0.6	0.2	0.5
		0.3	0.3	0.0
		2.5	2.5	1.5
		2.1	1.5	1.0
		1.2	0.8	1.0
Mean		1.1	1.0	0.6
St. Dev.		0.8	0.7	0.5
p value by t-test (N = 13)		0.02	0.06	0.27

Abbreviations

ABS	; Absorbance	Q.	; Quercus
abv	; Alcohol by volume	R^2	; Correlation Coefficient
amu	; Atomic mass unit	SPN	; Spanish oak
ANOVA	; Analysis of variance	St. Dev.	; Standard Deviation
cm	; Centimetre	SWRI	; Scotch Whisky Research Institute
EXP	; Exponential		
FID	; Flame ionization detector	UK	; United Kingdom
g	; Gram	USA	; American oak
GC	; Gas chromatography	UV	; Ultraviolet
GC-MS	; Gas chromatograph mass Spectrometer	yo	; Year old
		°C	; Degree Celsius
HPLC	; High performance liquid chromatography		
i-	; Iso-		
JPN	; Japanese oak		
L	; Litre		
m	; Metre		
mg	; Milligram		
min	; Minute		
mL	; Millilitre		
mm	; Millimetre		
n-	; Normal-		
N.D.	; Not detected		
P	; Probability value		

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